PHOTOCONTACT DERMATITIS

An investigation into photocontact dermatitis induced by benzanthrone.

by

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DECLARATION OF ORIGINALITY

I declare that the text of this thesis has been composed entirely by myself. Also, I have undertaken all the experimental work described within, with the exception of some assistance with the *in vitro* techniques and technical help in the analyses of blood ascorbic acid and renal function studies.

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ABSTRACT

The history of photocontact dermatitis caused by a variety of materials, mostly observed in an industrial setting, is reviewed. A modern approach to the problem, making use of current knowledge of photochemistry and photobiology is explained.

An outbreak of photocontact dermatitis attributed to the anthraquinone derivative, benzanthrone, and causing irritation and burning of the exposed skin, is described in chemical process operators working in a dyestuffs manufacturing factory. The chemistry of the supposed causative agent, and the manufacturing process involved are outlined.

The photocontact dermatitis was investigated by patch and photopatch testing with a Xenon arc lamp and diffraction grating monochromator. The signs and symptoms of the photodermatosis were reproduced experimentally with benzanthrone, and the evidence obtained from the tests indicated the reaction was phototoxic rather than having an immunological basis. Action spectrum studies showed that the most effective wavelengths were around 390 nm, near to the absorption spectrum for benzanthrone.

The phototoxic potential of benzanthrone was confirmed by *in vitro* studies using the photosensitized killing of Candida albicans and photohaemolysis of human red blood cells as model symptoms.

The mutagenic potential of benzanthrone was investigated using an Ames test modified by irradiating the *in vitro* system with UV at about 390 nm, and found to be negative.

As previous animal studies had suggested that the phototoxic effect of benzanthrone might be ameliorated with ascorbic acid, volunteers were photopatch tested before and after mega dose

ascorbic acid therapy. A clinical and statistical assessment of the results confirmed highly significant beneficial effects; however, the side effects of mega dose therapy in some volunteers seem to preclude the regular usage of ascorbic acid as a prophylactic in the prevention of the phototoxic reactions.

INTRODUCTION

Including

1. PHOTOSENSITIVITY

2. PHOTOTOXICITY

3. PHOTOALLERGY

4. PATCH AND PHOTOPATCH TESTING

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6. BASIC PHOTOBIOLOGICAL PRINCIPLES

1. PHOTOSENSITIVITY

The term photosensitivity is a general one used in dermatology to describe any eruption caused by light and does not imply an allergic mechanism. When the pathogenesis of the reaction is known the dermatitis can be classified as phototoxic or photoallergic. For general clinical purposes, however, photosensitization may be employed loosely to denote any instance of enhanced sensitivity to ultra violet (UV) and visible radiation in the skin, whatever the basic photochemical mechanism and subsequent reactions.

Chemicals causing photosensitization may enter the body by being inhaled, swallowed, injected, or absorbed through the skin. Obviously the fate of chemicals swallowed may be complex; they may be broken down in the gut and after absorption may be metabolized and detoxicated. If photosensitivity is to occur, absorption and distribution in the tissues (especially the skin) must be efficient and detoxication and excretion less so.

Most photosensitizers have a relatively complex chemical structure: many are cyclic or polycyclic and many have an alternating double and single bond system. The action spectrum of a photosensitizer is a reciprocal plot of the effectiveness in eliciting the clinical reaction in vivo against wavelength. The spectrum obtained may coincide with the absorption spectrum of the active compound.

Blum (1938) suggested three criteria for the recognition of photosensitization by exogenous agents. The first concerned the conditions under which photozensitization occurred in the patient, the second involved the reproduction of the lesion in experimental subjects, and the third was essentially an analysis and synthesis of the first two.

Magnus (1976) modified Blum's (1938) three rules in the light of recent technical advances to read:

1 (a) The symptoms and signs of photosensitivity must be elicited in the patient by irradiation whilst the agent is active.

(b) The action spectrum, drug dose-response and radiation dose-response should be found.

2 (a) The symptoms and signs must be reproduced in experimental subjects from the pure chemical by irradiation.

(b) The action spectrum and dose-response relationships should be found as above.

3 Action spectra and dose-response curves in (1) and (2) must be compared. If they appear to be similar, a case for photosensitization may be made. If they do not agree, reasons for this should be sought, should photosensitization still be entertained.

The pathogenesis of drug-induced photosensitivity has been reviewed by Levene & Magnus (1969), Harber & Baer (1972), Jarrat (1976) and Magnus (1976).

2. PHOTOTOXICITY

During the twentieth century may reports of phototoxic reactions followed the introduction of new therapies such as Eosin in the 1920's, sulphonamides in the 1940's, and tetracyclines in the 1950's. Phototoxic agents may also be found in foods, drinks, drugs, toiletries and cosmetics, plants, clothing, and in components of industrial materials and by-products. Some substances are phototoxic only after ingestion or injection, but others, including coal tar preparations, anthracene, psoralens and phenothiazines, are phototoxic after topical application. In general the action spectrum for phototoxic responses is similar to the absorption spectrum of the inciting compound.

Phototoxicity occurs in a large percentage of the population upon first exposure to the compound providing conditions, such as sufficient intensity of light and the quantity of photosensitizing chemicals, are appropriate. Phototoxic responses may take the form of delayed reactions similar to sunburn comprising erythema and occasional blisters followed by hyperpigmentation, but may consist simply of an immediate smarting and erythema.

The mechanism of phototoxicity seems to depend on (a) the binding of the chemical to various molecules in skin cells; (b) the absorption spectrum of the chemical and (c) the intensity and spectrum of the light to which the skin is exposed. Allison et al (1966) demonstrated that there were at least two types of phototoxic damage in vitro. If the agent bound itself to the cell membrane, exposure to light caused damage to the membrane with leakage of cytoplasm; however, if the agent concentrated in the lysosomes, light exposure caused lysosomal rupture and cell death. A third major cellular target site for the action of photosensitizers is nuclear DNA in which case, cell death or mutagenesis may occur. Whether these primary events occur in the

epidermis or upper dermis is not known, but both sites may be implicated.

Metabolites of the drug might also be responsible for clinical phototoxic reactions. Stratigos & Magnus (1968) found that when mice were given sulphanilamide intradermally the action spectrum peaked at 400 nm, whereas the absorption spectrum of the drug in vitro had its peak at 260 nm with no absorption above 300 nm. One explanation for this disparity is that the photosensitization is due to a sulphanilamide metabolite with different absorption characteristics, rather than the parent compound. On the other hand Pathak (1965) observed that photosensitizing drugs such as sulphanilamides, sulphonylureas, phenothiazines, tetracyclines and griseofulvin were most effectively activated by wavelengths between 290 nm and 310 nm. This is the spectrum that most effectively induces erythema in normal human skin.

3. PHOTOALLERGY

There has been much speculation in assigning an immunological basis to photoallergy. Amos (1973) stated that the criterion for an immunological pathogenesis involved evidence for "specific immunological activation". This may be obtained by demonstrating (a) antigen-antibody interaction, or (b) antigen reaction with cells that are specifically "allergized". However the concept of photoallergy is not yet proven.

Interestingly, there is a lack of agreement in photobiological and dermatological circles as to which chemicals or drugs cause phototoxicity and which photoallergy. Clinically, it may be difficult to distinguish between a phototoxic and a photoallergic reaction but the criteria in Table 1 (taken from Baer & Harber, 1971) may be helpful in distinguishing between the two types.

It is said that photoallergy is much rarer than phototoxicity, but Magnus (1976) concluded that prevalence, in part, depended on the method of provocation rather than the type of response.

Magnus (1976) also stated that clinical impressions, rather than hard scientific facts, appeared to be the basis of assertions that a photoallergic reaction required much less drug or chemical and radiation than a phototoxic reaction. Photoallergy was reviewed by Epstein (1977) and Frain-Bell (1979).

Table 1 Comparison of Phototoxic and Photoallergic Reactions

Reaction	*Phototoxic*	*Photoallergic*
Incidence	Relatively high (theoretically 100%)	Low (but could theoretically reach 100%)
Reaction possible on first exposure	Yes	No
Incubation period necessary after first exposure	No	Yes
Length of incubation period	Often 6 hours after first exposure	5 to 21 days
Chemical alteration of photosensitizers	No	Yes
Clinical changes	Exaggerated sunburn. Sometimes blisters. Pigmentation may be marked	Varied morphology. Usually very little pigmentation
Clinical symptoms	Often burning and smarting. Usually little itching	Itching common and may be severe
"Flares" at distant previously involved sites possible	No	Yes
Recurrence from exposure to ultraviolet alone	No	Sometimes in persistent light reactors
Can persistent light reaction develop?	No	Rarely
Cross-reactions to structurally related agents	Possible	Frequent
Broadening of cross-reactions following repeated photopatch testing	No	Possible
Concentration of drug necessary for reaction	High	Low
Action spectrum	Usually similar to absorption spectrum	Usually higher wavelength than absorption spectrum
Passive transfer	No	Possible
Macrophage inhibition	No	Possible
Lymphocyte stimulation test	No	Possible
Histopathology	Predominantly epidermal	Allergic inflammatory reaction in epidermis or cutis

4. PATCH AND PHOTOPATCH TESTING

Patch testing is a method of investigating skin reactions and has certain established fundamentals. Magnusson & Hersle (1965) investigated the most suitable site for patch tests by comparing reactions on the upper and lower back, on the flexor surfaces of the upper arm and forearm and on the extensor aspect of the thigh. The strongest responses to both irritants and allergens occurred on the upper back, probably due to the pressure of clothes and the weight of the body when lying down.

It is preferable when patch testing to utilise areas that are usually covered by clothing so that strongly positive reactions, which may persist for a week or so, will not embarrass the patient. For patients who are in hot, humid climates or who perspire so profusely that the adhesive does not adhere to the skin, the arms rather than the back may be used as the site and the patch tests held in place with a bandage.

When selecting test substances one should choose relevant substances with which the patient is in contact. However, it is often difficult to find out which these are, as the most common allergens are spread throughout the environment. For these reasons it has become common in most countries to use a standard test series (battery). Testing with a standard series may not be sufficient in itself and might have to be supplemented with additional substances in accordance with the patient's case history.

The concentration of the test substances is of fundamental importance for the test results. Standard test substances in routine concentrations rarely provoke irritant reactions, but false positive reactions occur occasionally. Most substances are patch tested at

concentrations between 0.1% and 1.0%

The patch test unit consists of a 10 mm central disc of filter paper fixed to a larger piece of impermeable inert material. The Al-test unit used in photopatch testing in this thesis consisted of a 1" square of aluminium foil covered with polythene with a 10 mm central disc of filter paper adhered by heat rather than by glue.

Patch tests are applied in strips and taped onto the upper back in vertical rows, avoiding the skin over the vertebral column. It is essential to mark the patch test sites, otherwise identification of positive reactions is impossible.

The patch test reaction depends on the sensitivity of the patient, the concentration of the substance, and the length of time it remains on the skin. Usually the patch tests are kept in place for 48 hours and 96 hours then removed and readings made. The actual reading should be made 20 to 60 minutes after removal of the patch so that the skin may recover from the effects of pressure which may produce transient mild erythema or even dermographia. The specific reaction to the test agent persists for hours or days.

In conventional photopatch testing the chemicals are applied to the skin in the usual way as for plain patch tests, but in duplicate. The exact procedure used after this may vary. In one version, all patches are removed after two days and the sites re-examined for a skin response. One of each set of the duplicates is re-covered, the others are irradiated. After irradiation these patches are once more covered. Another two days later all patches are removed and the reactions under them are noted. Reaction in the surrounding skin is also looked for and recorded. To be certain of differentiating between a contact dermatitis and a photocontact dermatitis the non-irradiated series should be kept constantly covered with black

material from the time of application to the final reading.

The variations in procedure are mainly concerned with how long the test materials remain in contact with the skin. For photopatch tests, this may depend on the chemical nature of the photosensitizer and the reaction it is thought to produce. For any thorough investigation of a suspected photosensitizer, the time of contact with the skin required to produce a positive response should be studied. Photocontact dermatitis alone gives a response in the irradiated patch only; contact dermatitis produces responses to both irradiated and non irradiated patches; contact plus photocontact dermatitis again gives responses to both but the irradiated shows a stronger response. It is important when recording patch test results that these are scored according to the observed reaction and not according to the interpretation placed on the reaction by the reader.

5. LIGHT SOURCES

The light sources used in photopatch testing are of three types; gaseous discharge, fluorescent and thermal. Spectral emission from the energy source should include that part of the spectrum which co-incides with the suspected action spectrum of the chemical under investigation. Such a source may be mercury discharge, which has the advantage of producing short ultra violet (UV) radiation if this is required, but has the disadvantage of having an interrupted emission spectrum. Even high pressure mercury discharge lamps produce a line spectrum superimposed on a continuous emission. Special low pressure mercury arc fluorescent tubes with selected phosphor linings of the glass envelope may be useful in that the emission spectrum may match the absorption spectrum of the photosensitizer. However, in many cases, the energy output from these lamps is too low to produce a photosensitized skin reaction with a reasonable exposure time. Carbon arc sources produce a continuous spectrum but their main disadvantage is that the electrodes, which are consumed after the arc is struck, need continual adjustment in order to emit energy at a constant level. Xenon arc discharge emits energy in a continuous spectrum, though this contains a higher proportion of short UV and infra red irradiation than does solar radiation. However, with the addition of suitable filters to correct these two discrepancies, Xenon arc emission more closely resembles solar radiation than any other known source and therefore seems to be the source of choice. Moreover, the use of cut-off filters will allow some estimation of the effective wavelength range for the skin reaction under study.

When, however, a light source is required to estimate the unknown action spectrum of a chemical which causes photocontact

dermatitis, a monochromatic light source has the obvious advantage of greater selectivity of narrow wavebands for irradiation of the photopatch test sites.

6. BASIC PHOTOBIOLOGICAL PRINCIPLES

Electromagnetic radiation describes a wide range from the shortest cosmic rays, through gamma rays, X-rays, ultra violet radiation, visible radiation, infra red radiation, and includes microwaves and all other wavelengths of radio energy. The division of this continuum of wavelengths (or frequencies) into a number of named sub portions is rather arbitrary. Nevertheless, to each of the commonly identified sub portions there correspond characteristic types of physical systems capable of emitting radiation of these wavelengths. Thus, gamma rays are emitted from the nuclei of atoms as they undergo any of several types of nuclear rearrangement; visible light is emitted, for the most part, by atoms whose planetary electrons are undergoing transitions to lower energy states; infra red radiations are associated with characteristic molecular vibrations and rotations; and radio waves, broadly speaking, are emitted by virtue of the accelerations of free electrons as, for example, the moving electrons in a radio antenna wire.

The visible range of the spectrum consists of electromagnetic radiation of wavelengths covering the range 400 to 700 nm. Radiation below 400 nm is invisible and lies in the ultra violet region, that above 700 nm is also invisible and lies in the infra red region.

Figure 1 illustrates the electromagnetic spectrum and identifies the characteristic regions. Visible radiation is indicated by the shaded area and represents about 75% of the solar radiation which may affect the human skin.

400 - 700 nm (visible light)

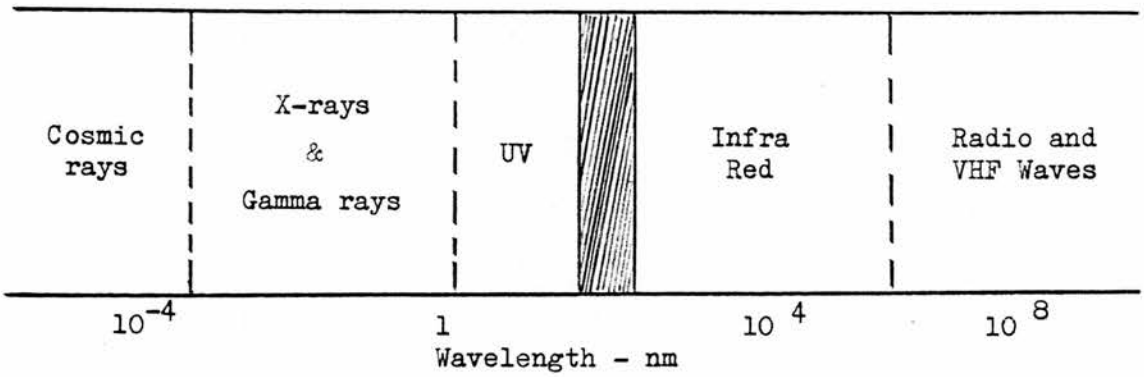


Fig. 1

Nearly all photosensitizing organic molecules that are stable contain an even number of electrons. When a molecule of this sort absorbs visible or ultra violet radiation, an electron is excited to one of the unoccupied orbitals of the molecule. The electron spins usually remain unchanged during the process of excitation and the molecule is said to be in the singlet excited state. In some instances, the singlet excited state which is very short lived (10^{-9}-10^{-8}sec), may be converted to a new state if the spin of one of the electrons is inverted and becomes parallel. The new state being described as the triplet state. The triplet state is usually long lived (10^{-3}-1.0 sec). Decay of the singlet excited state to the ground state is seen as fluorescence, and decay of the triplet to ground state as phosphorescence. The triplet state plays an important role in the transfer of energy and initiation of biological changes involved in photosensitization. Many dyes and other chemical molecules undergo transition to an excited state on exposure to light.

Photosensitising molecules in the excited state can transfer absorbed energy to biological molecules present in the system. Pathak (1969) described the following concept:

1. Transfer of energy from singlet excited state

$$P_{So} \xrightarrow{h_{vr}} P_{S1}$$

$$P_{S1} + B_{So} \longrightarrow P_{So} + B_{S1}$$

2. Transfer of energy from triplet excited state

$$P_{So} \xrightarrow{h_{v}} P_{S1}$$

$$P_{S1} \xrightarrow[\text{Conversion}]{\text{Intersystem}} P_{T1}$$

$$P_{T1} + B_{So} \longrightarrow P_{So} + B_{T1}$$

P_{So}, P_{S1} and P_{T1} respectively, indicate the ground state, the singlet excited state and the triplet state of the photosensitising molecule; B_{So}, B_{S1} and B_{T1} respective, indicate the ground state, the singlet excited state and the triplet state of the biological molecule. This transference of energy is most efficient when the absorption spectrum of the biological material, B, (the acceptor) overlaps the emission spectrum of the photosensitizer, P.

The excited electron states of molecules which are produced by absorption of visible or ultra violet light are deactivated rapidly by a number of mechanisms. One method is thermal decay, in which the energy of excitation is converted to translational, rotational and vibrational energy and distributed among all molecules in the system. A second method is by emission of light (e.g. fluorescence and phosphorescence). Unless the energy of this emitted light is transferred to other molecules in the cell, no photosensitization can occur.

Cutaneous photosensitization is produced by the transference of

energy from photoexcited molecules to constituents of cutaneous cells, or to the oxygen contained in them, with consequent denaturation or destruction of cellular components. Almost all of the agents implicated in the photosensitization of biological systems are known to be fluorescent either in solution or when adsorbed on a surface.

Factors affecting photosensitization include:

1. Absorption spectra of photosensitizing agents and skin:

The basic photochemical law of Grotthuss & Draper states that "only radiation that is absorbed by a reacting system is able to produce chemical change". Absorption involves the transfer of energy to the system. The absorption spectrum of the photosensitizing molecule usually determines the action spectrum of photosensitization, but in a heterogeneous system such as skin, the action spectrum may be modified by binding of the agent with cellular constituents or by preferential localization of the agent in certain layers.

2. The structure of photosensitizing molecules:

Molecules that are composed of tricyclic condensed aromatic rings in linear arrangement with or without hetero-atoms (e.g. O, N, S) are known to sensitise biological systems to light and a small amount of photic energy only is needed to activate them. Many of these photosensitizing molecules contain nitrogen and oxygen atoms, and have a lone pair of electrons which are not involved in binding.

3. Concentration of the sensitizing agent:

The concentration of a photosensitizing agent in a given biological system may determine the degree of photosensitivity evoked.

4. The wavelength and energy content of impinging light:

The energy content varies inversely with wavelength, e.g. the energy content of the quantum corresponding to 300 nm is twice as great as that corresponding to 600 nm. Because the specific energy

differences in biomolecules which might provide a target for skin reactions such as sunburn coincide with the photon energies of wavelengths around 300 nm and below, it is this region of the spectrum which is responsible for such reactions. Any seasonal variation in photosensitization in skin due to longer wavelengths may be due to the fact that although these wavelengths are prominent throughout the year, they are, of course, also more intense during the summer months. Cutaneous photosensitization occurs most commonly in the Northern Hemisphere between April and September as the intensity of ultra violet radiation is greater then than in the winter months.

5. The erythemal threshold of the subject:

The tolerance of the skin to radiation within the sunburn spectrum (290 - 320 nm) is primarily governed by the thickness of the stratum corneum and by the melanin content of the melanocytes and keratinocytes. These factors may also affect photosensitization.

6. Temperature:

The absorbed radiation may increase the temperature of the absorbing system and thus alter the rate of biological photosensitization. True photochemical reactions have practically no temperature coefficient because their energy of activation is derived from absorption of light and not from thermal activation. However, the effectiveness of methylene blue in sensitising certain biological systems to light was shown by Oginsky et al (1960) to be increased by elevation of the temperature of the system.

7. Hydrogen ion concentration (pH):

Alterations in the pH affect the photosensitizing capacity of an agent by altering the cationic and anionic character of both the sensitizing agent and the biological material being sensitized.

8. Effect of oxygen:

Blum (1941) showed that photosensitization of biological systems by such agents as methylene blue, acriflavin, eosin and other dyes was oxygen dependent. He termed such reactions as "photodynamic".

Chapter 11

A HISTORICAL APPRAISAL OF PHOTOBIOLOGICAL ACTIVITY IN POLYCYCLIC, AROMATIC AND HETEROCYCLIC COMPOUNDS.

Including

1. THE CLASSIFICATION AND NOMENCLATURE OF CARBON COMPOUNDS
2. TAR AND PITCH
3. TEXTILE DERMATITIS
4. ULTRA VIOLET CURED INKS
5. EPOXY RESINS
6. SALICYLANILIDES AND RELATED COMPOUNDS
7. SYSTEMICALLY ADMINISTERED DRUGS
    a) Sulphanilamide
    b) Oral Contraceptive Agents
    c) Phenothiazines
    d) Tetracyclines
8. BENZANTHRONE

1. THE CLASSIFICATION AND NOMENCLATURE OF CARBON COMPOUNDS

Classification

By the broadest system of classification, carbon compounds fall into three main divisions:

1. ALIPHATIC

Aliphatic, fatty or open chain compounds, in which the carbon atoms, when more than one is present, are linked in such a manner that no closed rings containing carbon are formed. They may be saturated, when each carbon atom is linked to one or more others by single bonds, or unsaturated, when one or more double or triple links between carbon atoms will be present.

2. CARBOCYCLIC

Carbocyclic compounds in the molecules of which closed rings of carbon atoms are present. Benzene is the simplest representative of the aromatic carbocyclic compounds and contains a six membered ring possessing peculiar stability and reactions. The aromatic compounds which include not only benzene derivatives but all compounds containing one or more aromatic rings, whether separate or fused with other carbocyclic rings, form a very large and important class.

3. HETEROCYCLIC

Heterocyclic compounds having in the molecule at least one ring containing, besides carbon atoms, one or more atoms of another element or elements such as oxygen, nitrogen or sulphur.

Nomenclature:

The aim of the system of organic nomenclature is to give to every compound a name which, without ambiguity, conveys the exact constitution of the compound, enabling its formula to be written down at once. The convention according to which every organic compound can be regarded as a derivative of a parent hydrocarbon forms the basis of the system of nomenclature.

2. TAR AND PITCH

Although the photosensitivity reactions induced by epoxy resins, dyestuffs and components of the ink curing process are clearly examples of industrial or occupational photodermatoses, the majority of reactions in this class have been due to tar and pitch.

The first person to describe a disease primarily influenced by light was Robert Willan. In his morphologic arrangement of skin diseases compended by Bateman (1818), eczema was placed in the general category vesiculae. Recognising that direct rays of the sun accounted for some cases of eczematous eruptions occurring during the summer months, Willan introduced the term "eczema solare".

Volkman (1875) seems to have been the first to note occupational dermatitis due to pitch and tar when he described cutaneous changes due to these substances. Soon after, Manouvriez (1876) described pigmentation in workers exposed to fumes from hot tar and dust from pitch. Ehrmann (1909) later extended these observations and recorded three types of cutaneous reaction to pitch; (1) dermatitis with hyperkeratoses and papillomas; (2) pitch comedones and folliculitis and (3) melanosis. He aptly designated the last "Indianerhaut" but failed to recognise photosensitization as its cause although he noted that the condition was confined to the face and neck and the exposed parts of the arms.

Lewin (1913) was probably the first to associate pitch with a photodynamic effect. He observed a hundred cases of dermatitis among the employees of a cable works engaged in impregnating paper tubes with hard coal tar pitch; the condition was confined to the exposed parts and included all stages of the dermatitis reaction, and was associated with burning and itching that were worst when the patient went into the sunlight. He did no experimental work, but concluded

that the dermatitic reaction might be a contact dermatitis or be due to the effects of absorption, either directly through the skin, or by the inhalation of vapour of a photodynamic substance.

Herxheimer & Nathan (1917) reported a photosensitive dermatitis due to "Carboneol", a solution of coal tar in carbon tetrachloride and chloroform. This condition was an acute papular vesicular dermatitis occurring as sharply demarcated elevated oedematous plaques on light-exposed areas only. Ultra violet rays were suspected as the cause, but again no experimental work was carried out.

Hoffman & Habermann (1918) described pigmentation of the skin in patients who had been in contact with tars, oils and greases. They suspected that heat or light played some part in either the causation or intensity of the pigmentation.

Bettman (1918) noted post inflammatory pigmentation of parts exposed to light in workers handling wartime grease substitutes, and similar cases were described by Oppenheim (1916).

Koelsch (1919) and Ullman (1926) wrote extensively on the occurrence of dermatoses in tar and naphtha distilling industries. They recorded that the two distillates caused similar dermatoses despite their fundamental chemical differences. Koelsch described the intermittent character of the inflammatory reactions of pitch and asphalt workers and observed that the signs and symptoms of erythema, oedema and pruritus were confined to exposed parts and were less severe after diffuse light exposure compared with direct sunlight or light reflected from snow.

McCafferty (1927) described a case of mottled erythema and pigmentation involving exposed parts of the forearms in a patient handling a creosote-containing substance outdoors in the sun.

O'Donovan (1929) observed pigmentation in outdoor workers who handled crude anthracene but noted that men packing the distillates indoors were unaffected.

Uhlman (1927) showed that erythema followed by pigmentation occurred commonly in briquet, tar and anthracene workers in the Ruhr. The incidence was higher in the summer months and mainly affected day shift labourers. Similar cases produced by pitch and oils were also described by Ravaut & Vibert (1927), Mornet (1928) and others.

Kistiakowski (1930) reported a photosensitive dermatitis, pigmentation, comedones and folliculitis in workers involved in manufacture of artificial asphalt from coal tar, anthracene oil and coal tar pitch. He ascribed the photosensitization to inhalation of vapours and ingestion of dust containing anthracene, acridine and phenanthrene.

Wieder (1932) reported occupational pigmentation of the face in a chemist. He reacted to a variety of petroleum and coal tar derivatives used in the dyestuff industry when exposed to artificial ultra violet rays (nature unspecified) but exhibited no reaction when these rays were excluded.

Kinnear (1935) reported facial pigmentation in a jute spinner associated with oil folliculitis of the arms, forearms and legs. He described the condition as being similar to that reported by Hoffman & Habermann (see page 24).

Tar preparations have been used therapeutically in dermatology for many years. Dioscordes described the use of asphaltic tar as a panacea for cutaneous disorders nearly 2,000 years ago (Downing & Bauer 1948). Coal tar was first discovered and described by Becher & Serle in 1681, but it was not until two centuries later that Fischel,

in 1894, mentioned details of its application. It had been alleged that coal tar contained a photosensitizing element which made the skin more sensitive to ultra violet radiation resulting in increased erythema and pigmentation. Goekerman in 1925 published his regimen for the treatment of psoriasis by means of crude coal tar and ultra violet radiation. He published a further account of this treatment in 1931. Since the introduction of this mode of therapy it has generally been accepted that the effect has been due to photosensitizing substances in coal tar.

Herrick & Sheard (1928) showed that exposure of crude coal tar to ultra violet radiation resulted in photochemical changes which could be detected by changes in the absorption spectrum of the irradiated tar.

Fleischhauer (1930) conducted experiments on the photosensitizing effects of coal tar when applied to the skin. When the coal tar preparation (Lianthral) was applied to skin and subsequently exposed to an erythema dose of ultra violet radiation, the resultant erythema was more intense than when no tar was used. Although a more pronounced effect was noted under noon day summer sun, a similar result was obtained when a mercury vapour lamp was used.

Obermayer & Becker (1935) studied the effects of crude coal tar and allied substances on the skin in conjunction with ultra violet radiation. They used crude coal tar and distillation products of the crude tar which they divided into the high, middle and low temperature fractions. They found that the high temperature fraction was the most effective of the distillates in treating psoriasis, but was not as effective as the crude coal tar. They postulated that the efficacy of this regime was due to a photosensitizing element in the coal tar which was acridine.

During 1961 Everett, Daffer & Coffey, using a hot quartz mercury vapour lamp, failed to confirm Fleischhauer's findings, probably due to the variation of the output from the lamp and the differing absorption characteristics of the Liquor Picis Detergens which was used.

Foerster & Schwartz (1939a & b) investigated an outbreak of dermatitis and pigmentation among 500 men in four factories involved in the manufacture of electric conduits where pitch was used extensively. The most severe dermatitis and most intense pigmentation were observed in men employed outdoors cutting, polishing and stacking finished conduits. Some workers were so severely affected that they could not be employed in the daytime but worked satisfactorily on the night shift. The authors noted that pitch in powder form was more likely to cause burning and pigmentation than any other physical form. A variety of materials were used for patch testing and photopatch testing using air cooled and water cooled mercury vapour quartz crystal burners with a spectral emission of 185 - 320 nm, but predominantly at 310 nm; a mercury vapour tungsten filament lamp with a spectral emission of 188 - 253.7 nm, but predominantly at 200 nm and below; a carbon arc lamp with a spectral emission of 300 - 430 nm, but mainly around 380 nm; and natural day light on cloudy and sunny days. No positive photopatch test results were obtained using these artificial light sources, and after studying the absorption spectra of various chemicals known to be present in tar, they concluded that the ultra violet wavelengths responsible for the dermatitis and melanosis were between 390 nm and 500 nm. They also concluded that variations in response could be attributed to varying sources of radiation. They observed that pitch which had previously been exposed to sunlight for an hour, 6 months or 4 years did not produce positive

patch test responses, nor did it produce positive responses after ultra violet or carbon arc irradiation. They concluded that exposure to light did not change the photobiologic activity of pitch by changing it chemically or physically. (As mentioned on page 26 Herrick & Sheard, 11 years earlier, postulated that irradiation of coal tar produces chemical change).

Crow et al (1961) described an outbreak of photosensitivity in workers in a factory in which various articles were moulded from a hot mixture of pitch, asbestos and slate dust. The investigation showed that the photosensitivity reactions included the "smarts", a term used to describe the intense stinging and burning sensation often associated with erythema; in most cases it preceded the erythema but in a few cases it occurred without erythema at all. The smarting started within a quarter of an hour of exposure to the sun light, but disappeared in 1 to 2 hours after moving into the shade, though occasionally it persisted for 12 hours. Most men found that window glass offered no protection suggesting that the action wavelength must have been above 320 nm. Indians and negroes were unaffected. It was only possible for them to study one employee from the factory, though they confirmed their findings in three normal volunteers. The problem was investigated by taking advantage of the greater waveband selectivity of an irradiation monochromator with a Xenon arc source. Forearm skin, painted with a pitch and chloroform mixture twice daily for 3 days, gave rise to a burning sensation within 5 to 10 minutes of being irradiated with light at 10 nm intervals over the range 320 - 450 nm. This was followed by strong erythema lasting for at least 48 hours. Control skin gave no such results.

Chlorpheniramine Maleate (Piriton), 12 mg and 24 mg orally, had no influence on the urticarial reaction and smarting produced with

anthracene and 360 nm irradiation in two subjects. The authors postulated that the reaction was not therefore mediated by histamine, and that in this respect it resembled the sunburn response (Partington 1954). Crow and his colleagues also stated that the urticarial response observed could not be described as a triple response, as unlike the observations of Foerster & Schwartz, a flare was not seen. They then investigated the role of oxygenation of the skin. Forearm skin which had been previously prepared with pitch or anthracene was irradiated for 15 minutes, 5 minutes after the application of a sphygmomanometer cuff on the upper arm inflated above systolic pressure. This gave no abnormal skin response. The experiment was then repeated a few minutes later with normal blood flow, when smarting and urticarial reaction occurred, and the results were confirmed in two other subjects. Their inference, that this reaction was likely to be a photo-oxidative one, is open to some doubt as constriction of a limb with a sphygmomanometer does not remove the oxygen completely but merely reduces it (Naylor, 1960). Therefore if pitch and anthracene photosensitivity is due to a photodynamic action, the reduction of skin oxygen tension by the cuff is evidently sufficient to interfere with the development of lesions associated with smarting.

Gotz (1976) examined 111 former workers in the tar industry and found 71% to be affected with the effects of tar and pitch exposure. He concluded that these materials accelerated the normal skin-ageing process and also observed that the changes occurred more on those areas exposed to sunlight (face and dorsa of hands). Eighty two per cent of the tar workers still showed increased ultra violet light sensitivity at the time of determination of their threshold ultra violet erythema values despite an average non exposure time to tar of 8.8 years. Gotz postulated that photosensitizing tar derivatives

remained in the epidermis and caused erythematous reactions to ultra violet radiation for a prolonged period.

Kaidbey & Kligman (1977) found that the phototoxicity of coal tars diminished with refinement, and that the least active of the tars studied was liquor carbonis detergens. Long ultra violet radiation (UV-A), following skin painting, elicited a reaction which occurred in two stages, an immediate weal and burning followed, in one to two days, by a red infiltrated plaque and persistent post-inflammatory pigmentation.

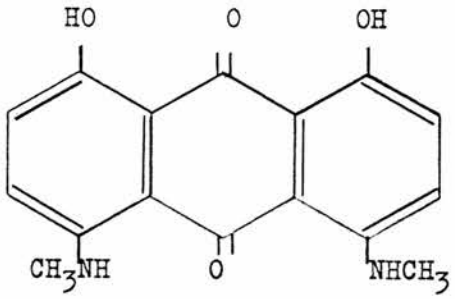
Emmett et al (1977) investigated two groups of "roofers" exposed to coal tar pitch. Six of 34 men in one group and 11 of 17 in the other had clinical signs of keratoconjunctivitis. Those affected complained of severe burning of the eyes on exposure to the sun. In addition the majority of "roofers" in each group gave a history of several episodes of probable keratoconjunctivitis in the past which they related to exposure to pitch vapours or dusts. The eye and skin symptoms were clearly associated with the use of coal tar pitch, rather than other roofing materials, and were more pronounced when pitch was used at a higher temperature. As a result of their observations, Emmett et al embarked on a controlled study of the effects of ultra violet radiation and pitch alone, and in combination, on the eyes of New Zealand white rabbits. Preliminary experiments showed that rabbits, irradiated from a bank of BLB 40 fluorescent black lights (predominantly 330 - 380 nm) passed through a window glass filter to remove radiation below 320 nm shortly after conjunctival installation of 10 µl of coal tar pitch distillate to both eyes, would tightly close only the irradiated eye within about 3 minutes (4×10^2 joule/m^2UV) and keep that eye closed until irradiation ceased. Two rabbits were killed 24 hours after treatment with pitch and subsequent irradiation.

Histological examination of their eyes, showed marked congestion of the palpebral conjunctiva and extensive sloughing of the corneal epithelium with early superficial keratitis. No changes were observed in the iris, lens or other ocular structures. Eyes treated with pitch alone showed oedema and early cell necrosis of the corneal epithelium only. Eyes treated with radiation alone were histologically normal. Their studies therefore indicate that exogenous photosensitizers such as coal tar pitch components can cause phototoxic damage to the cornea and conjunctiva and that this can be assessed in animal models.

Interestingly, Susorov (1970) also noted that eye irritation in pitch workers was more pronounced when work was carried out in clear sunlight.

It is surprising that pitch has so rarely been incriminated as a cause of persistent light reaction, despite its ability to produce photodermatitis. However, Barefoot (1979) has recently described a patient in whom the history, positive photopatch tests to pitch, histological findings and otherwise negative laboratory findings supported a diagnosis of persistent light reaction to pitch.

3. PHOTOTOXIC TEXTILE DERMATITIS



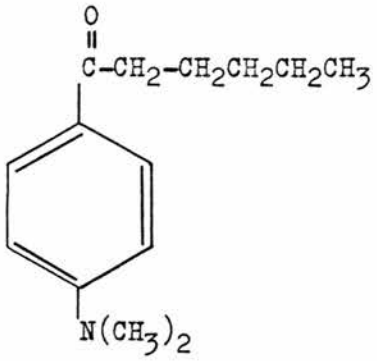
Disperse Blue 35

Fig. 2

Hjorth & Møller (1976) reported two cases of phototoxic textile dermatitis from bikinis. The first woman complained that after sunbathing in a new brown bikini she developed a burning erythema on her back under the brassiere strap. She washed the bikini but developed identical symptoms when wearing it again. The skin pigmented to form a brown band across her back. The second patient had brown triangles on her chest corresponding with the shoulder straps of her black and white bikini. Patch tests, performed only on the first patient, elicited no reaction. After extraction of the dye from the bathing suits, fifteen fractions could be visualised by two-dimensional thin layer chromatography. Two of these fractions are found in Disperse Blue 35, an anthraquinone dye which Gardiner et al (1972) demonstrated caused photocontact dermatitis in a dye manufacturing process. The fractions identified elicited photopatch test reactions in healthy volunteers. A chromatogram of Disperse Blue 35 gave fractions similar to those obtained from the extracts of the brown bikini, but not of the black one.

Interestingly, Keith-Davies and colleagues (1975) had shown that the main photochemical reaction of Disperse Blue 35 was that of efficient production of singlet oxygen which caused the oxidation of both tryptophan and lecithin.

4. ULTRA VIOLET CURED INKS



Amyl p-Dimethylaminobenzoate

Fig. 3

Ultra violet (UV) cured inks are a relatively recent development but their use is becoming widespread. The ink composition varies but usually consists of one or more conventional pigments dispersed in a polymeric vehicle. The vehicle usually contains the following: various polyfunctional acrylic monomers; a UV-reactive unsaturated polymer; one or more photo-initiators; diluents; hydrogen transfer agents; and a variety of miscellaneous agents as stabilisers, fillers, surfactants and polymerisation inhibitors.

The first step in the curing process is the absorption of UV radiation by the photo-initiator. The absorption of UV results in the generation of free radicals or other photo-excited states which in turn cause polymerisation of the vehicle in which the pigments are incorporated and thus cure the ink film. Medium pressure mercury arc lamps are generally used for the cure.

Emmett et al (1977) described an investigation into photo sensitivity caused by UV cured inks. Four white men, aged from 26 to 52 years, employed in weighing, mixing or milling UV-cured inks complained of sensitivity to sunlight. The symptoms and signs included burning (smarting) on exposed areas, erythema and swelling. There was evidence of eczematous dermatitis in two of the men,

apparently due to allergic contact sensitization to certain acrylates used in the UV-cured ink formulation. In order to determine which of the materials used in the UV cured ink formulation might cause photosensitization, the UV absorption spectra of all ingredients were determined in ethanol using a double beam spectrophotometer. Six photo initiators used in UV cured inks were found to absorb UV radiation above 250 nm. These were benzophenone, thioxanthone, 2-2'-diethoxyacetophenone, 4,4'-bis dimethylamino benzophenone (Michler's ketone), and two different commercial preparations of industrial grade mixed isomers of amyl dimethylaminobenzoate, designated as absorber A and absorber B.

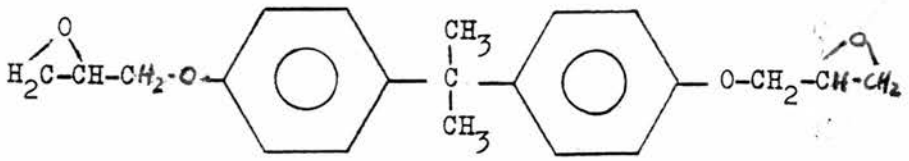
Photopatch testing was performed on 3 employees who complained of photosensitivity, and 4 employees free of photosensitivity, using benzophenone (5% in petrolatum); diethoxyacetophenone (5% in petrolatum); thioxanthone (5% in petrolatum); Michler's ketone; absorber A (5% in petrolatum); absorber B (5% in petrolatum); absorber A and absorber B. Two duplicate sets of patches were applied to the back. One set of patches was removed from each of 7 subjects 24 hours after application, and the areas gently cleansed with 70% ethanol in water. These sites were then exposed to sunlight. Four employees were exposed to 25 minutes of clear noon sunlight and 3 employees to 35 minutes of sunlight from 1550 hours. Reactions were observed after the irradiation. The second set of patches, that functioned as a dark control for the development of allergic contact or irritant reactions, was removed 48 hours after application and reactions at both sites were read and graded one hour later.

The majority of subjects complained of a sharp stinging or burning sensation within a few minutes of sun exposure localised to the exposed areas where absorber A and absorber B had been applied.

Twenty four hours after sun exposure, 6 of the 7 subjects had a reaction consisting of uniform erythema and oedema on the area exposed to undiluted absorber A and sunlight; 2 of the 7 men also had reactions to undiluted absorber B under the same conditions. No reactions were seen to undiluted absorber A or B on sites occluded from sunlight and no reactions were seen with the other UV ink components phototested either on exposed or occluded sites. No reactions were seen on sun exposed or control sites tested with either absorber A or absorber B diluted to 5% in petrolatum.

The authors concluded that the two industrial preparations of mixed isomers of amyl dimethylaminobenzoate designated as absorber A and absorber B were phototoxic, but the action spectrum was not delineated. The responses were probably phototoxic because of the relative immediacy of the response to sunlight, the intense burning sensation on exposure to sunlight, the ability to reproduce the reaction in other employees not complaining clinically of photo-sensitivity, as well as in previously unexposed persons, and the failure of reactions to occur when these substances were diluted.

5. PHOTOSENSITIVITY FOLLOWING OCCUPATIONAL EXPOSURE TO EPOXY RESIN



Epoxy resin monomer

Fig. 4

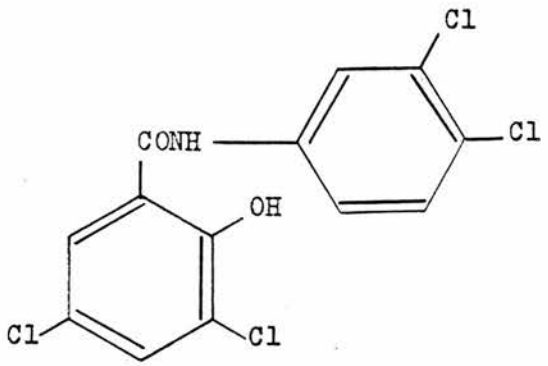
Allen & Kaidbey (1979) reported 8 cases of persistent photosensitivity in men following occupational exposure to hot epoxy resin fumes. The 8 men worked as pipe fitters or foremen for a local gas and electric company, and had all previously used epoxy resins in their work without trouble. In January 1978, difficulty arose in securing a good seal around leaky gas pipes with the epoxy compound they were using. Part A, the epoxy resin, consisted of 88.5% 4-4-isopropylidine diphenol epichlorohydrin epoxy resin and butyl glycidal ether epoxy reaction diluent; Part B, the catalyst, consisted of 68.2% polyamines, 15.5% coal tar, cresylic acid, phenols, and 16.3% fillers. The epoxy resin was heated to 60°C prior to mixing it with the catalytic reagent in order to obtain a better sealing effect. However, on puncturing the heated cans, dense fumes were released that contacted uncovered skin sites. Two weeks later, an acute papulovesicular eruption of the face, neck, dorsal aspect of hands, and distal part of the forearms developed in 3 men. Patch tests with 1% epoxy resin from a patch test tray revealed strongly positive delayed vesicular reactions in all three. There was no further trouble until the Spring (about 2 months later) when all 8 men noted, in areas previously exposed to the hot resin fumes, the sudden onset

of stinging, burning and erythema following exposure of 10 to 15 minutes to either direct sunlight or light filtered through window glass. The photo dermatitis improved over a period of 2 to 3 weeks but never cleared completely.

The patients were photopatch tested with 1% of mixture A; 1% epoxy resin from the patch test tray; and 4-4 isopropylidenediphenol (1%, 0.1% and 0.01% in hydrophilic ointment respectively). The 3 patients who had given a positive patch test to epoxy resin were first patch tested with tenfold dilutions of the resin (0.1%, 0.01% and 0.001% respectively) in hydrophilic ointment. The highest concentration that failed to elicit a reaction 72 hours later was chosen for photopatch testing. For the photopatch testing, a 150 W Xenon solar simulator, equipped with a filter, was used to provide a continuous ultra violet light spectrum from 320 - 400 nm. The results of the photopatch tests showed that the same 3 patients who had given positive patch tests to the 1% epoxy resin from the patch tray also had positive photopatch tests to the diluted resin, and hence had a combined contact and photocontact sensitivity to epoxy resin. One other patient showed photocontact sensitivity to epoxy resin.

It is interesting that the phototoxicity in these patients started in the Spring with the increase in sunlight intensity and that the phototoxic reactions were strictly localised to areas exposed to resin fumes. One year after the onset of the photosensitivity, in spite of strict avoidance of further exposure to the agent, the men remained exquisitely sensitive to sunlight and had to remain indoors during the daytime hours. They can therefore be classified as persistent light reactors.

6. SALICYLANILIDE AND RELATED COMPOUNDS



3,3',4,5'-Tetrachlorosalicylanilide

Fig. 5

Phenols and their derivatives have been used as skin disinfectants since the beginning of the century, but rapid strides were made after the development of polyphenolic compounds in 1943. The compounds found to be most useful had two benzene rings connected by a bridge; at least one of the rings carried halogen atoms and one or more phenolic OH groups. Substitution of salicylanilide with groups other than halogens or modification of the salicylanilide structure usually diminishes the activity as a germicide.

Tetrachlorosalicylanilide (T.C.S.A.) was used in many countries for a short time because of its excellent antibacterial action, but its impressive sensitizing and photosensitizing capability soon became apparent causing its withdrawal from use in soaps.

T.C.S.A. is a chemically stable, white crystalline, non hygroscopic, odourless, tasteless powder, melting at 161° - 163°C. It is sparingly soluble in water; the weakly acidic character (pH 6.6) of the phenolic group accounts for its ready solubility in alkaline solutions. It has a characteristic ultra violet absorption spectrum with peak absorption at 360 nm in ammoniacal methanol. In acidic

methanol the peak shifts to a shorter wavelength. T.C.S.A. fluoresces when excited by UVR.

Wilkinson (1961) described the first fifty three cases of photodermatitis due to incorporation of T.C.S.A. in two new soaps. The incidence of sensitivity was so high that 29 workers in a factory of 106 men exposed to the soaps developed contact dermatitis. The onset was dramatic and in many cases occurred during the weekend or after exposure on a bright day. Marked male predominance was noted, suggesting that men washed more vigorously or that this particular type of soap was used more frequently by men. The eruptions involved areas of the body exposed to light, such as the hair parting, bald areas of uncovered heads, lobes of ears, double chin protuberances and uncovered legs. The dorsa of the hands were less frequently and less severely affected. The erythema developed swiftly, progressing in severe cases to exudation, and finally desquamation, leaving dry, tight, lichenified and later cracked and pigmented skin. Wilkinson cast suspicion on T.C.S.A. because of its known fluorescence. Standard patch tests carried out by Wilkinson were strongly positive in 16 out of 22 patients and mildly positive in 3, using 0.25% or 1.0% T.C.S.A. in alcohol; 6 out of 7 patients reacted to 0.1%, but tests on 4 patients who had recovered from the initial attack proved negative at 0.1%. No reaction occurred in 26 out of 27 normal controls. Some delayed reactions were observed and strong reactions were still visible two weeks later. Wilkinson suggested that a degradation product of T.C.S.A. could be produced in the presence of light and might be the cause of the clinical condition. Incorporation of T.C.S.A. in soap in England commenced in July 1960 and was stopped in October 1960, largely on account of an approach made by Wilkinson to the manufacturer.

Soon after Wilkinson's first description, other reports appeared in England. Calnan et al (1961) described 102 patients (87 men and 15 women) with a similar clinical picture. Ninety gave positive patch tests to T.C.S.A. In a group of 74 patients, 26 had a positive reaction to a closed patch test with 1% T.C.S.A. In the rest, a positive reaction could be produced only by exposure to T.C.S.A. on the test area to a suberythema dose of ultra violet radiation. Control tests on persons who had not had previous contact with T.C.S.A. were negative. Several patients had a persistent dermatitis (up to 4 months) with acute relapses occurring on further exposure to sunlight, even in the absence of continued contact with the soap.

In 1962, Wilkinson reported a further 50 cases and again found positive reactions to T.C.S.A., and also on this occasion to tribromosalicylanilide (T.B.S.). No reactions were observed in 32 control patients patch tested with 1% T.C.S.A., 25 with 1% T.B.S., 14 with 1% trichlorocarbanilide (T.C.C.) and 12 with 1% trichlorosalicylanilide (TriC.S.A.). "Straightforward primary sensitisation" was the presumed diagnosis. Cross sensitivity was recognised among the group of substances T.C.S.A., T.B.S., T.C.C. and dibromosalicylanilide (D.B.S.). An impurity or oxidation or degradation product of T.C.S.A. was suggested as the possible cause of the rash.

At about this time, soaps containing T.C.S.A. were being field tested in America but these were discontinued when reports of the difficulties in Britain appeared. Vinson & Platt (1962) found that 8 known T.C.S.A. photosensitive patients were still strongly reactive one year or more after last using the soap. Photopatch tests with other salicylanilides showed wide cross sensitivity. However, no cross-reactivity was noted to T.B.S. indicating to the authors its

relative safety. These negative results were different to those of Wilkinson and may have been due to the low concentration (0.04%) of the compound used in the patch tests.

Several instances of contact dermatitis to bithionol had been reported by Gaul in 1960 and 1963, one patient showing probable cross sensitivity with hexachlorophane. Jillson & Baughman also reported 4 cases of photocontact sensitivity to bithionol in 1963, but none reacted to hexachlorophane. These authors emphasised that if only suberythema doses of ultra violet radiation were used in photopatch testing, most cases of bithionol photocontact dermatitis would be missed.

Baughman (1964) added 13 more patients to the growing list of persons photosensitive to bithionol. Of these, 4 were patch test positive but not photopatch test positive to hexachlorophane. Two also showed positive patch tests to T.B.S. In 1965 Epstein & Enta described two middle-aged patients who were photosensitive to T.B.S., presumably due to the use of a soap containing this compound. Both of these patients had stronger photopatch tests to T.C.S.A. than to T.B.S. After 1965, the frequency of reports of photosensitivity to salicylanilides increased rapidly. Molloy & Mayer in 1966 reported photosensitivity to D.B.S. in two middle-aged patients, and in the same year Epstein reported three cases of contact sensitivity to dichlorophane. Harber and colleagues (1966) described 3 patients sensitive to several bacteriostatics. O'Quinn et al (1967) described 20 patients suffering from photocontact dermatitis due to bithionol, and 3 with a similar condition due to hexachlorophane in soaps and cosmetics, both occurring with equal severity in white and negro patients. Burry, in the same year, described transient and persistent photo-allergies to

the antifungal preparation Multifungin (5 bromo 4' chlorosalicyl-anilide), which is chemically very similar to T.B.S. and T.C.S.A. and to fenticlor (bis [2 hydroxy 5 chlorophenyl] sulphide), which is chemically very similar to bithionol. Cross reactions between fenticlor and hexachlorophane and between fenticlor and bithionol were reported as were those between multifungin and T.B.S.

Willis & Kligman (1968) noted the persistence (up to 6 months) of salicylanilides in the affected skin of patients showing prolonged light sensitivity to these agents. They excluded the epidermis as the tissue depot because it was entirely replaced in about one month. They thought that the compounds might precipitate out in the dermal interstices and remain there due to the extreme insolubility of salicylanilides in aqueous solutions and their great binding power.

7. PHOTOSENSITIVITY TO SYSTEMICALLY ADMINISTERED DRUGS

Many drugs have been incriminated in the induction of photosensitivity reactions and in their comprehensive review written in 1942, Stokes & Beerman cited five drugs as photosensitizing; sulphonamides, barbiturates, gold, silver and arsphenamine. When Kirschbaum & Beerman re-examined the subject in 1964 they quoted about 50 systemically administered drugs as being photosensitizing agents. They divided these into five main groups: (1) sulphonamides and their derivatives (e.g. anti-diabetic agents and thiazide diuretics), (2) phenothiazines, (3) tetracyclines, (4) furocoumarins (psoralens) and (5) miscellaneous, including griseofulvin, antihistamines and many others. Photosensitivity eruptions have also been reported to nalidixic acid (Zelickson 1964; Baes, 1968; Birkett et al, 1969) and cyclamates (Lamberg, 1967).

Only the major ones will be discussed here.

Oscar Raab (1900) was probably the first to demonstrate the deleterious effects of light on a living organism in the presence of a chemical agent. He found that suspensions of living Paramecium Caudatum in normally non-toxic concentrations of acridine dye were killed when exposed to light. He appreciated that the photobiologic response was due to an obligative combined effect of both dye and light. Each alone was without effect, but the combined effect of photosensitizer and appropriate wavelengths of light was lethal to the cell.

Meyer-Betz (1913) showed that human skin could also be photosensitized by a chemical substance. He injected himself with 200 mg of haematoporphyrin. Sunlight caused gross oedema and erythema of his face which took several weeks to settle.

(a) Sulphanilamide

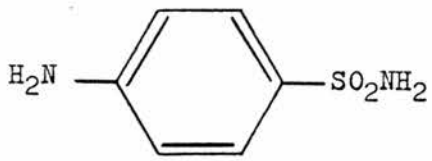
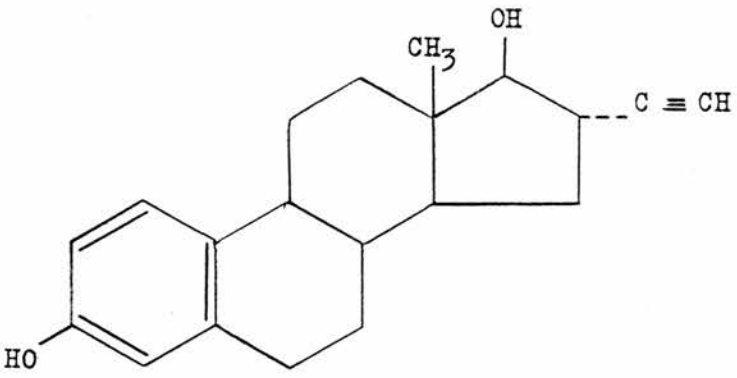


Fig. 6

Epstein (1939) irradiated sites of intradermal injections of sulphanilamide with a mercury vapour arc. All of his six subjects developed a marked erythematous reaction at the injection site within 24 hours. The reaction lasted several days and left increased pigmentation. However, 2 of the 6 subjects also had a further episode of erythema at the original site 10 days following irradiation. Accordingly, Epstein separated the two types of reaction to irradiation into (1) primary photosensitivity (photodynamic action) and (2) allergic photosensitivity (photoallergy). Blum (1941) confirmed Epstein's findings, but challenged his use of the expression "photodynamic action" which, he said, should refer precisely to an oxidative reaction produced by light requiring the presence of molecular oxygen, whereas the sulphanilamide response to light was evidently not so dependent. Subsequently the term "phototoxic" was employed for this response.

Burckhardt (1941) carried out a similar investigation by injecting 7 volunteers with sulphanilamide (1%) and irradiating them with sunlight, ultra violet (300 - 400 nm) and visible light ($>$400 nm). A positive reaction was seen at all the sites.

(b) Oral Contraceptive Agents



Ethinyloestradiol Ethynylestradiol; 17-ethinylestradiol

Fig. 7

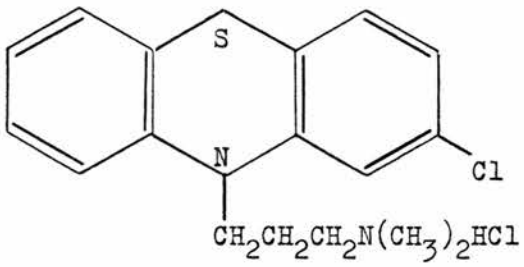
Mathison & Haas (1970) reviewed the literature from 1950 to 1969 on skin conditions associated with the use of oral contraceptives. They discussed the three major cutaneous reactions to oral contraceptives as (1) chloasma, (2) porphyrias, and (3) true photosensitivity. Under the last heading they included the report of Erickson & Peterka (1968) of the first photosensitive reaction due to an oral contraceptive.

Oosterhuis (1968) also reported a photosensitivity reaction induced by an oral contraceptive; however, in contrast to the data of Erickson & Peterka he was unable to demonstrate any cross photosensitization when the patient was given another type of oral contraceptive.

Ortho-novin (Norethisterone and Mestranol) was implicated as the agent responsible for the production of severe lesions on the hands and fingers in a study by Baxter & Permowicz (1967). The lesions occurred on exposure to sunlight after taking the oral contraceptive for six months. Discontinuation of the drug, avoidance of sunlight and the use of a sun screening agent caused remission.

Elgart & Higdon (1971) reported a similar case to that of Erickson & Peterka. This involved a 23 year old Caucasian Peace Corps worker who, with her husband, was transferred to Western Samoa. The patient was in good health, used no soaps or cosmetics containing photosensitizers and gave no history of previous light reactions. Porphyrin studies and a lupus erythematous cell preparation were negative. Her facial photosensitivity reactions commenced three months after commencing treatment with ortho novin (norethisterone and mestranol).

(c) Phenothiazines



Chloropromazine Hydrochloride

Fig. 8

De Eds et al (1940) showed that workers involved in spraying phenothiazine, a tricyclic compound used as an insecticide, developed eruptions on light-exposed areas. They found that oral phenothiazine caused photosensitivity, but that topical application did not, and therefore concluded that the untoward effects of phenothiazine were due to its systemic absorption via the respiratory tract.

The tranquillizer phenothiazine chlorpromazine, was first used in France in 1952 and since that time has been widely prescribed. As a result of contact with chlorpromazine, a dermatitis localized to face, hands and fingers may develop on exposure to normal or excessive sunlight. This takes the form of a scaly erythema and is associated with burning and itching.

Winkelman (1957) reported that 3% of 1090 patients treated with moderate to large doses of chlorpromazine developed photosensitivity. Cahn & Levy (1957) found that the wavelengths between 296 - 302 nm were chiefly responsible.

Calnan and colleagues (1962) studied chlorpromazine contact dermatitis and investigated 180 employees over a 6 year period who were involved in its manufacture in a pharmaceutical company. All

were patch tested and 125 were accepted as cases of chlorpromazine dermatitis. One hundred and four were patch test positive and 21 were negative. Sixty cases were also photopatch test positive.

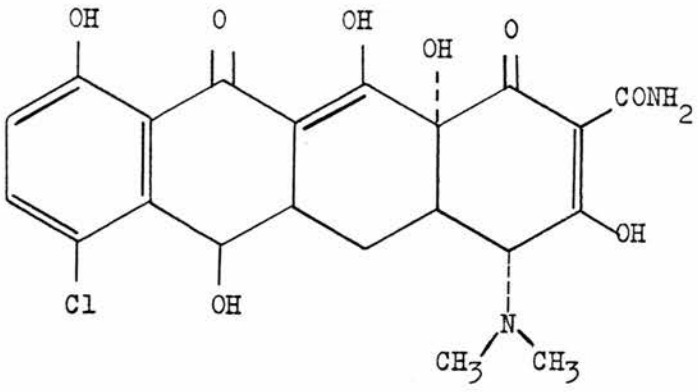
Hunter, Bhutani & Magnus (1970) investigated the photosensitizing action spectrum of chlorpromazine in hairless mice. Their results suggested that chlorpromazine photosensitivity had a broad action spectrum in the range 330 - 380 nm, with peak activity at wavelengths just above normal sunburn wavelengths.

The haemolysis of red cell suspensions has been used as a model to examine photosensitization due to chlorpromazine, and using this system, Johnson (1974) found that chlorpromazine photohaemolysis had a dose-response relationship to the concentration and radiation.

Patients photosensitized by receiving chlorpromazine, and nurses by handling it, were investigated by Raffle and his colleagues (1975) but, using the lymphocyte transformation test, they were unable to distinguish between a phototoxic and a photoallergic reaction.

Ljunggren & Möller (1977) investigated the phototoxic potential of twenty seven commercial tricyclic drugs in vivo using a mouse tail technique capable of quantification. Here, after systemic administration of the test drug, the tails of the animals were immediately exposed to long ultra violet light (UVA). The degree of phototoxic inflammation was calculated on the basis of the increase in wet weight of the mouse tail. The most potent drugs were chlorpromazine and two other chlorinated compounds, prochlorperazine and perphenazine. Tricyclic drugs lacking nitrogen, sulphur, or both in their ring system showed no activity. The capacity of different drugs to inhibit the outgrowth of Candida albicans exposed to UVA was also studied in vitro and the results confirmed the animal studies.

(d) Tetracyclines



Demethylchlortetracycline
7-Chloro-6-demethyltetracycline

Fig. 9

There has been disagreement on the action spectrum for tetracycline photosensitivity. Some early reports suggested that in human subjects ultra violet radiation (UVR) below 320 nm was responsible (Cahn et al 1961). However, more recent studies in humans, and in experimental animals, have implicated longer wave UVR ($>$ 320 nm) and visible light (Stratigos & Magnus, 1968; Ison & Davis 1969). Segal (1963) reported the case of a 60 year old man who, after taking 2g of tetracycline hydrochloride daily for a week, developed severe sunburn when on a fishing trip. Frank, Cohen & Minkin (1971) reported a similar exaggerated sunburn reaction in a woman and a boy. Epstein et al (1976) reported porphyria-like cutaneous changes in 5 patients exposed to strong sunlight while taking tetracycline hydrochloride for acne vulgaris.

Demethylchlortetracycline (DMCT) is the most phototoxic of the tetracyclines. Cahn, Levy & McMillen (1961) showed that white men taking 600 mg daily became photosensitive, but, if the dose was reduced to 450 mg daily, they experienced amelioration of their symptoms. This phototoxic effect was minimal in coloured men. Blank,

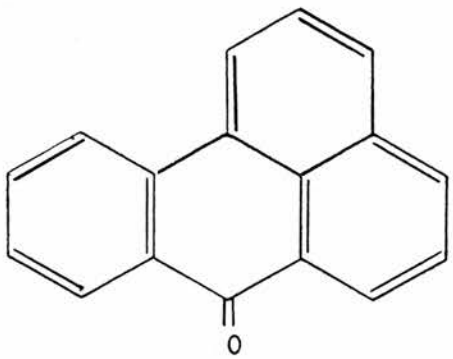
Cullen & Catalano (1968) gave 10 white volunteers 600 mg of DMCT daily for one week and then took them sailing on a sunny day. Within 5 hours, 7 developed a phototoxic reaction followed by 2 more within 2 days. The phototoxic reaction was sufficient to require administration of systemic steroids to 7 subjects.

Harris (1950), in New York, reported that 2 men out of 135 patients with brucellosis given chlortetracycline 2.5 - 4.0g daily, developed severe phototoxicity requiring withdrawal of the antibiotic. Similarly, Verhagen (1965) reported that 10 out of 63 children given 15 mg per kilo body weight developed an increased susceptibility to sunburn during bright sunny weather.

Frost, Weinstein & Gomez (1972) noted that 11 out of 15 volunteers given doxycycline 200 mg daily for one week developed a phototoxic reaction while on a day's sea trip.

Tromovitch & Jacobs (1963) studied a 20 year old man who remained photosensitive for 6 months after taking oxytetracycline 2g daily for 3 days. Photopatch testing showed that he reacted to the sunburn spectrum (290 - 320 nm).

8. BENZANTHRONE



Benzanthrone

Fig. 10

Benzanthrone, an anthraquinone derivative, is the starting product for an important group of vat dyes. The term "vat dye" describes a colouring matter, insoluble in water, which can be converted on reduction into a leuco compound soluble in alkalis; in such a solution, termed a "vat", the actual dyeing process is effected. The vat dyes owe their properties to the presence in the molecule of two keto groups. The dyes consist of polycyclic structures, containing up to nineteen condensed rings, which are built up from anthraquinone or its derivatives. They are almost entirely used for dyeing cotton and related cellulosic fibres as viscose rayon, and they are the fastest of all known colouring matters to light.

Wieder (1932) described a research chemist who, over a twelve year period, had been exposed to various chemicals, including chloro-benzanthrone and benzanthrone, used in the manufacture of dyes and vat dye intermediates. The first problems noted were mild redness, dryness and itch of the face and neck, followed by pigmentation of the

exposed areas. The benzanthrone, to which the patient was exposed, contained 5% anthraquinone as an impurity and patch and photopatch tests to this impure material produced moderate erythema at the unirradiated site followed by faint pigmentation lasting several months, and severe erythema followed by pigmentation lasting several months, at the irradiated site. Patch and photopatch tests to benzanthrone produced similar but milder results. Patch and photopatch tests to chlorobenzanthrone and anthraquinone were negative. Wieder concluded that the nature of the reaction and the results of investigations indicated a definite photosensitizing effect.

Trivedi & Niyogi (1968) reported an investigation carried out in a dyestuff manufacturing factory in India where cases of blackening of the skin had been reported. During benzanthrone manufacture from anthraquinone, the workers were exposed to powdered benzanthrone at the drying, pulverising and packing stages. Seven workers from the 48 examined were patch tested on the forearms for one week with pure benzanthrone and pure anthraquinone. The patch tests gave negative reactions. An identical series of patch tests with similar negative results was carried out on one of the authors. Photopatch testing using a UV light source (unidentified) with pure benzanthrone and pure anthraquinone was then carried out daily for 4 minutes over three consecutive days on the same investigator giving a negative result again. The authors stated that the negative reactions to the patch and photopatch tests were most likely due to an insufficient period of exposure both to the chemicals and the UV light.

Interestingly, air sample analysis for benzanthrone dust from the manufacturing plant showed a concentration of benzanthrone of 630 mg/m^3. The American Conference of Governmental Industrial Hygienists Handbook, to which the British Government mainly adheres,

gives a threshold limit value of 10 mg/m^3 for nuisance dust and so the Indian workers were exposed to extremely high levels of the dust.

Singh & Zaidi (1969) reported pruritus, erythema and pigmentation in 25 Indian dye workers exposed to benzanthrone. The affected workers were males aged 22 - 60 years. The reactions started as burning and itching which became unbearable after exposure to sunlight, and was associated with pigmentation. Two of the workers complained of watering of the eyes. The authors did not carry out patch or photopatch testing and merely observed that exposure to benzanthrone appeared to cause the phototoxic effect.

Benzanthrone Toxicity

Acute oral LD50 (rat) > 500 mg/kg)Unpublished studies carried out
)at ICI Central Toxicology
Acute dermal LD50 (rat) > 1000 mg/kg)Laboratories in 1971.

Intraperitoneal LD50 (rat) 1500 mg/kg)National Institute for Occupational
)Safety & Health, Registry of Toxic
Intraperitoneal LD50 (mouse) 290 mg/kg)Effects of Chemical Substances 1979.

Singh, Sharma & Zaidi (1967) studied the direct effect of benzanthrone on the skin of mice. Four groups of 10 mice were studied as follows :-

Group 1 2% benzanthrone suspension in normal saline was applied daily to the shaved dorsal surface and exposed to sunlight one hour per day for 40 days.

Group 2 Dorsal skin shaved and exposed to sunlight one hour per day for 40 days.

Group 3 2% benzanthrone suspension in normal saline was applied daily to the shaved dorsal surface but the animals were excluded from sunlight.

Group 4 Normal controls.

Two animals were sacrificed from each group at 10 and 20 days and three animals at 30 and 40 days.

Results

One animal in Group 1 showed slight thickening and roughness of the skin after 20 days which was more marked on the fortieth day. The rest of the animals in all the groups did not show any gross abnormality.

Histopathology

Group 1 Microscopy of the epidermis after 20 days revealed no specific changes. However, the dermis was oedematous and infiltrated with a few neutrophils and mononuclear cells. Microscopy on the fortieth day showed hyperkeratosis, a prominent granular cell layer and acanthosis. There was a marked increase of fibrous tissue, the blood vessels and capillaries in the dermis and subcutaneous tissue were dilated and there was a mononuclear infiltrate in the dermis. The follicles in the deeper layers showed proliferation of the germinal matrix cells which were degenerated in places. The sebaceous glands showed hyperplasia.

Group 2 No specific histopathological changes were seen.

Group 3 Microscopy after 20 days showed slight vasodilatation and infiltration with a few neutrophils and mononuclear cells.

Group 4 No specific histopathological changes were seen.

The authors concluded that benzanthrone in the presence of sunlight stimulated cellular growth in all the skin layers and acted as an irritant causing increased vascularity and fibrosis.

As Magos & Sziza (1962) and Salzberg (1964) had reported that ascorbic acid and riboflavin were effective in the prevention of allergy, sensitivity and toxicity of the skin due to various synthetic

dyes, Singh & Zaidi (1969) studied the effect of ascorbic acid and riboflavin on benzanthrone-induced skin lesions. The dorsal skin surfaces of 180 male albino mice were shaved and six groups of 30 animals were studied as follows :-

Group 1 Normal controls exposed to sunlight one hour each day for 40 days.

Group 2 5% benzanthrone suspension in distilled water was painted daily on the shaved surface and exposed to sunlight one hour each day for 40 days.

Group 3 5% benzanthrone suspension in distilled water was painted daily on the shaved surface and exposed to sunlight one hour each day for 40 days, and 50 mg/kg body weight of ascorbic acid was fed orally daily.

Group 4 5% benzanthrone suspension in distilled water was painted daily on the shaved surface and exposed to sunlight one hour each day for 40 days, and 50 mg/kg of riboflavin was fed orally daily.

Group 5 Animals fed 50 mg/kg body weight of ascorbic acid daily and exposed to sunlight one hour each day for 40 days.

Group 6 Animals fed 50 mg/kg body weight of riboflavin daily and exposed to sunlight one hour each day for 40 days.

Ten animals from each group were sacrificed at intervals of 10, 20 and 40 days.

Results

Group 1 Hair growth appeared normal. Microscopic examination of the skin showed normal appearance of all skin layers throughout the experimental period.

Group 2 Thick growth of hair was noticed up to 20 days followed by irregular thinning. At the end of 40 days the hair became

scanty, rough, dry and dirty brown in colour. Microscopic examination at 20 days revealed no specific changes in the epidermis but the dermis contained a few neutrophils and mononuclear cells. On the fortieth day there was irregular acanthosis and hyperkeratosis, again with a prominent granular cell layer in places. There was increased fibrosis in the dermis and some infiltration with mononuclear cells.

Group 3 Healthy growth of hair was evident throughout the experiment. Microscopic examination revealed slight hyperkeratosis on the fortieth day.

Group 4 No improvement in hair growth was noted. Microscopic examination showed marked acanthosis in one animal. After 20 days in the rest of the animals, there was slight vasodilatation and infiltration of the dermis with a few neutrophils and mononuclear cells.

Group 5 Hair growth appeared normal.

Group 6 Hair growth appeared normal.

The authors concluded that the oral administration of ascorbic acid (50 mg/kg) in experimental animals appeared to inhibit the dermal changes caused by the local application of benzanthrone. Riboflavin was ineffective when given in the same dose.

Since the experimental work of Singh & Zaidi (1969) suggested that ascorbic acid appeared to protect mice from the effects of benzanthrone, Pandaya, Singh & Joshi (1970) studied the toxic action of benzanthrone in scorbutic animals and the reversal of its toxic effect by ascorbic acid.

One hundred male guinea pigs were divided into four groups of 10 (1 - 4) and three groups of 20 (5 - 7) as follows :-

The animals in Groups 1 - 4 were kept on a routine stock diet.

Group 1 Normal control.

Group 2 25 mg/kg body weight of benzanthrone suspended in 1 ml normal saline was injected intraperitoneally daily.

Group 3 50 mg/kg body weight ascorbic acid dissolved in 1 ml water was fed orally daily.

Group 4 50 mg/kg body weight ascorbic acid dissolved in 1 ml water was fed orally and 25 mg/kg body weight of benzanthrone suspended in 1 ml of normal saline was also injected intraperitoneally daily.

Animals of all the groups were sacrificed after 7 days and 5 ml of blood was drawn from the hearts. The abdomen of each animal was opened and the liver and adrenals removed. Histochemical examination of ascorbic acid on one of the adrenals (left), and estimation of the total ascorbic acid content of the blood, liver and right adrenal were carried out. The results of histochemical examination showed that the adrenals belonging to Groups 1 and 3 showed cells densely filled with compact ascorbic acid granules. In Groups 2 and 4 however, the ascorbic acid granules were scanty. Ascorbic acid levels in the blood, liver and adrenals were significantly lowered ($P < 0.01$) in the benzanthrone treated animals of Group 2 as compared with the control Group 1. However, significant differences were not observed in the ascorbic acid contents of these tissues after administration of ascorbic acid in Group 3 as compared to the controls. Ascorbic acid supplementation in benzanthrone treated animals (Group 4) appreciably restored the blood ascorbic level ($P < 0.01$) compared with animals in Group 2.

The remaining 60 guinea pigs were the subject of a mortality rate study and were divided into three groups of 20 animals as follows :-

Group 5 were fed a routine stock diet.

Group 6 were fed a synthetic scorbutic diet.

Group 7 were fed a synthetic scorbutic diet with the addition of 25 mg/kg body weight/day of ascorbic acid.

After 15 days a stat dose of 500 mg/kg body weight of benzanthrone in normal saline was injected intraperitoneally into each animal of all the groups.

The results of the mortality study showed that animals in Group 5 on a normal stock diet had a mortality rate of 50%. Animals in Group 6 on a scorbutic diet had a mortality rate of 100% and animals in Group 7 on a scorbutic diet with added ascorbic acid supplements had a mortality rate of 60%. Scorbutic animals appeared to be completely unprotected from the toxic effects of benzanthrone due to the lack of ascorbic acid.

The experiments of Pandaya et al showed that administration of benzanthrone caused a significant decrease of ascorbic acid in the blood, adrenals and liver. Supplementation with ascorbic acid, while only having a slight effect on the levels in the adrenals and liver, restored the blood ascorbic acid levels. Similarly the mortality rate in Group 7 showed a 40% lowering after supplementation with ascorbic acid as compared with the scorbutic group (Group 6), emphasizing the protective role of the vitamin in cases of benzanthrone toxicity. Pandaya et al therefore postulated, that ascorbic acid might be used to prevent workers from developing toxic effects when exposed to benzanthrone.

Chapter III

NATURE OF THE PROBLEM

Including

1. HISTORY OF THE BENZANTHRONE PROBLEM AT GRANGEMOUTH WORKS

2. THE MANUFACTURING PROCESS

3. OUTLINE OF PROSPECTIVE INVESTIGATION

1. THE NATURE OF THE PROBLEM

Benzanthrone has been manufactured in Grangemouth Works of Imperial Chemical Industries (I.C.I.) for over forty years. During this period a significant number of employees engaged in its manufacture and packing have developed two apparently distinct skin conditions.

1. Photocontact dermatitis. The employees have complained that the sun causes irritation and burning of exposed benzanthrone-contaminated skin, and even erythema in more severe cases. Those parts most effected being the malar and frontal areas and the dorsal aspects of the hands. Plates 1 and 2 show good examples. The workers often have associated intense pruritus, usually localised to the anterior aspect of the upper thighs. The condition usually subsides in 24 hours but occasionally lasts 2 to 3 days. It is never a major clinical problem, in that it does not persist when chemical contact or sun exposure are avoided, but it could, if appropriate preventive measures were not taken, become a serious industrial problem. It is, however, a social problem in that employees cannot follow out-of-door pursuits when affected.

Whereas day shift craftsmen, supervisors and chemists have been affected, the highest incidence is among process workers who follow a 3 shift system of work i.e. 6.00am - 2.00pm (morning shift), 2.00pm - 10.00pm (back shift) and 10.00pm - 6.00am (night shift). The majority of cases among process workers have been observed on morning shift; skin discomfort developing soon after leaving the factory at 2.00pm on bright sunny days and even whilst on their way home. Evening sun caused symptoms only if it was exceptionally bright. Those morning shift workers who had burning and erythema in the afternoon were usually compelled to stay indoors, or at least out of direct sunlight,

Plate 1

Erythema on dorsal aspects of hands of employee photographed at the end of his shift and after contamination with benzanthrone and exposure to sunlight.

Plate 2

Photograph of employee at the end of his shift displaying marked erythema of malar and neck areas.

for several hours. The condition affected both the new and long term employees. Most affected personnel noticed that the sun made their skin react, even when it was transmitted through glass, for instance when they were driving their cars.

2. Contact sensitization dermatitis of an unusual nature has been observed in a small number of employees. It is not related to sunlight and is not seasonal. It is typified by a generalised morbilliform rash which is often symptomless. Plate 3 shows a good example of this rash. No treatment is required, but the employee must be removed permanently from any further contact with benzanthrone. Standard patch tests are always positive and as the sensitivity is often so exquisite that laundering of clothing is not sufficient to remove all traces of benzanthrone, the contaminated garments are destroyed. Over a five year period (1975 - 1980) only three workers have been removed permanently from the manufacturing shed due to this form of contact sensitization.

Although pigmentation of light-exposed skin has been reported from benzanthrone manufacturing workers in India (Trivedi & Niyogi 1968; and Singh & Zaidi 1969) it has never been observed in Grangemouth.

Workers at Grangemouth are supplied with cleanly laundered underwear, socks and boiler suits at the start of each shift. Full showering and changing facilities are available for all employees and are regularly used by all manufacturing shed personnel. In an attempt to lessen the burning effect of benzanthrone, a UVR barrier cream, containing mexenone (Uvistat), has been provided for several years with varying results, but no attempt has been made to ascertain the aetiology of the phototoxic reaction.

Plate 3

Generalised morbilliform rash in a process operator who developed allergic contact dermatitis to benzanthrone.

2. BENZANTHRONE - THE PROCESS OF MANUFACTURE

Benzanthrone

Introduction:

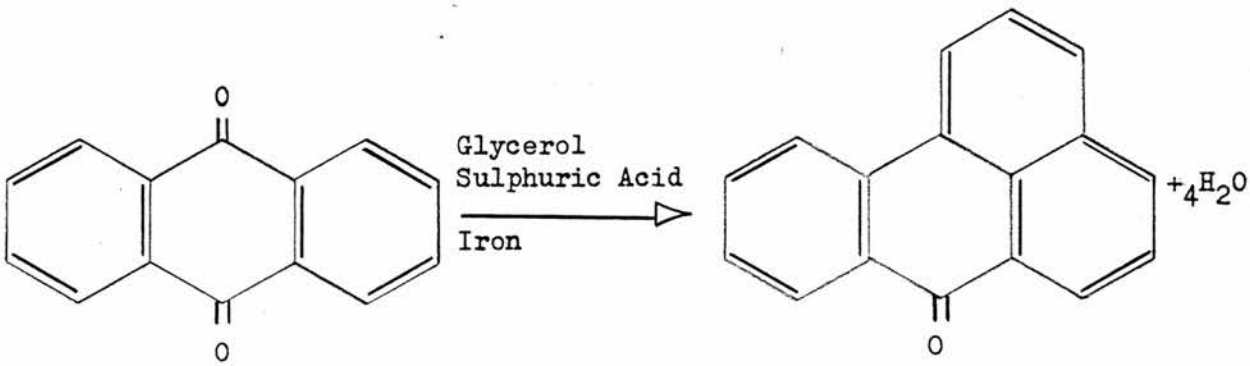
The manufacturing route is a "Skraup" reaction between glycerol and anthraquinone in sulphuric acid in the presence of iron. The process consists of two stages -

(a) Condensation and isolation of crude benzanthrone.

(b) Purification of the crude material by vacuum distillation.

Crude benzanthrone is produced by the simultaneous reduction/ condensation of anthraquinone in sulphuric acid using an iron and glycerol mixture. The reaction mass is diluted with water, filtered, washed acid free with hot water, and then dried in an agitated vacuum dryer.

The reaction is represented by :-



Anthraquinone (Mol. Wt. 208)

Benzanthrone (Mol. Wt. 230)

Fig. 11

The purification of the crude product is effected by vacuum distillation and the molten product cooled, flaked and pulverised.

a) Preparation of Crude Benzanthrone

Fig. 12 represents a schematic diagram of both stages of the process. Sulphuric acid is charged to a cast iron reaction pan containing an agitator as shown in Plate 4. The acid strength is then

adjusted by addition of water. The temperature in the reaction vessel is raised by applying steam to the pan jacket and the anthraquinone powder is charged through a charge door on top of the reaction vessel. The charge is further diluted and the temperature is further raised. An iron/glycerol mixture, previously prepared in a separate mild steel reaction pan, is then blown over to the reaction vessel at a controlled rate. When condensation is complete the charge is cooled and transferred to a dilution vessel and diluted with water, to give crude benzanthrone. The suspension of crude benzanthrone is then blown to the filter press shown in Plate 5, and the press is washed with hot water until its contents are acid free. When the filter press cake has been adequately washed the process operator separates the plates and must, by hand, drop the cake into paste boxes using a scraper. Although skin contamination can occur at this stage of the process it does not seem to give rise to photocontact dermatitis, presumably because minimal amounts touch the skin. The filtered benzanthrone paste is dried in a vacuum dryer, heated with low pressure steam, as shown in Plate 6, and the crude product, which is in the form of pebbles of uniform size ($\frac{1}{4}$"-$\frac{1}{2}$" diameter) is discharged to hoppers.

b) Distillation of the Crude Benzanthrone

The crude benzanthrone from the batches is charged to a mild steel still shown in Plate 7, using a pneumatic handling system and the still is sealed and heated. During heat up, bound water and air are removed via an ejector. Vacuum is applied at a suitable temperature over 1-2 hours and distillation to the mild steel vacuum receiver commences.

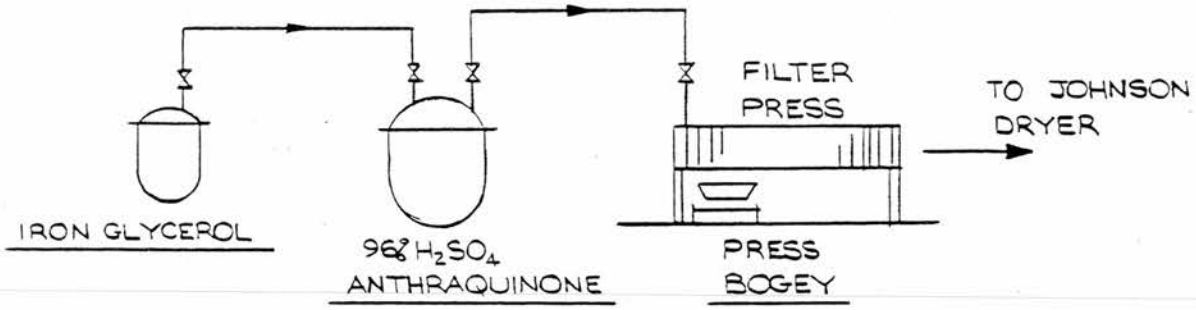
The distilled benzanthrone is transferred by gravity to a receiver, and the receiver contents are then passed continuously to

a bandcaster, shown in Plate 8, which cools and flakes the solidified benzanthrone. This particular part of the process requires constant adjustment by the process operator who is therefore exposed to vapour of benzanthrone. In addition, flakes of pure benzanthrone can escape from the side of the revolving hot plate of the bandcaster and these must be recovered manually. Considerable skin contamination can occur at this stage. The flaked material is fed directly to a mill, shown in Plate 9, and the pulverised material discharged into steel drums. Handling of such solid particulates is a universal problem in the chemical industry, and transferring the pulverised material into drums is another source of skin contamination.

Benzanthrone is therefore manufactured in two stages, crude and purified. At certain stages of the manufacture, physical handling is necessary and skin contamination can and does occur.

SCHEMATIC DIAGRAM OF BENZANTHRONE MANUFACTURE

1. CONDENSATION STAGE



2. DISTILLATION STAGE

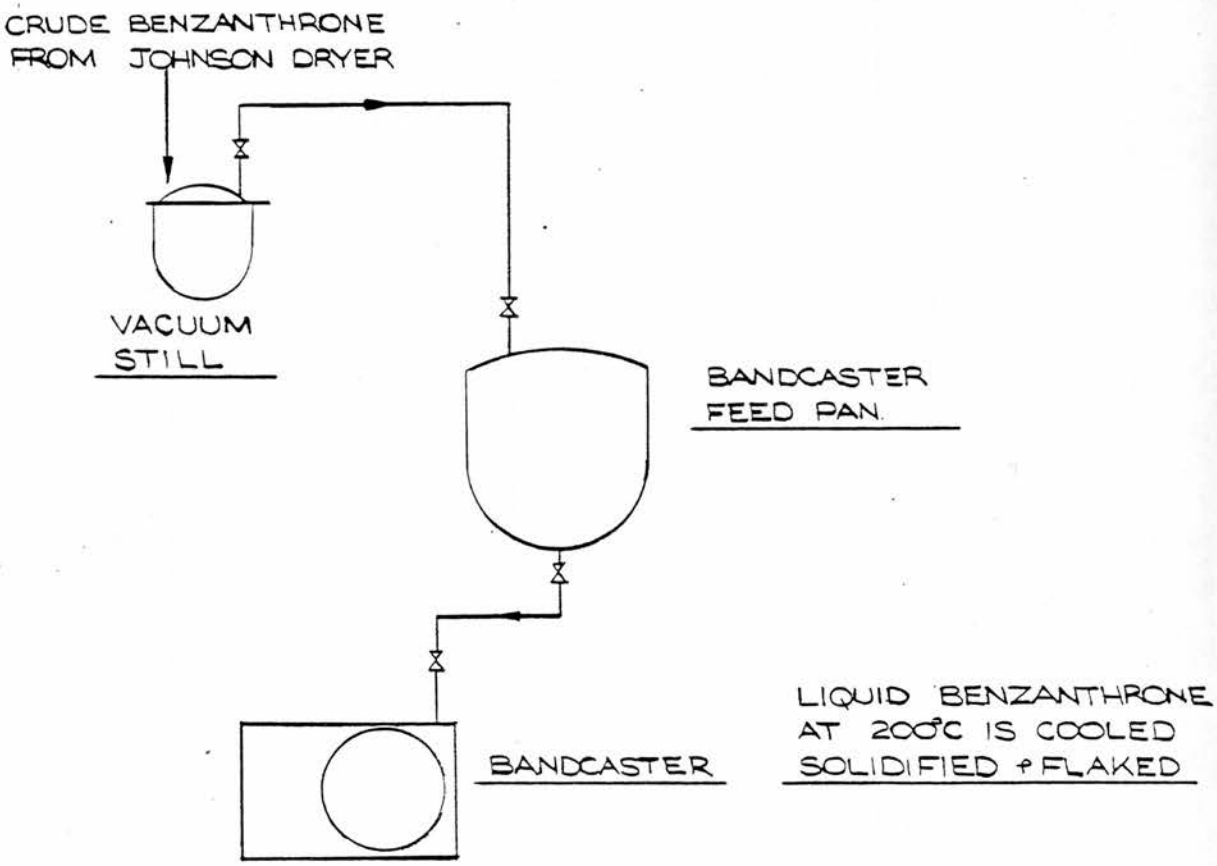


FIG. 12

Plate 4

Reaction vessel containing anthraquinone powder, sulphuric acid, and iron/ glycerol mixture.

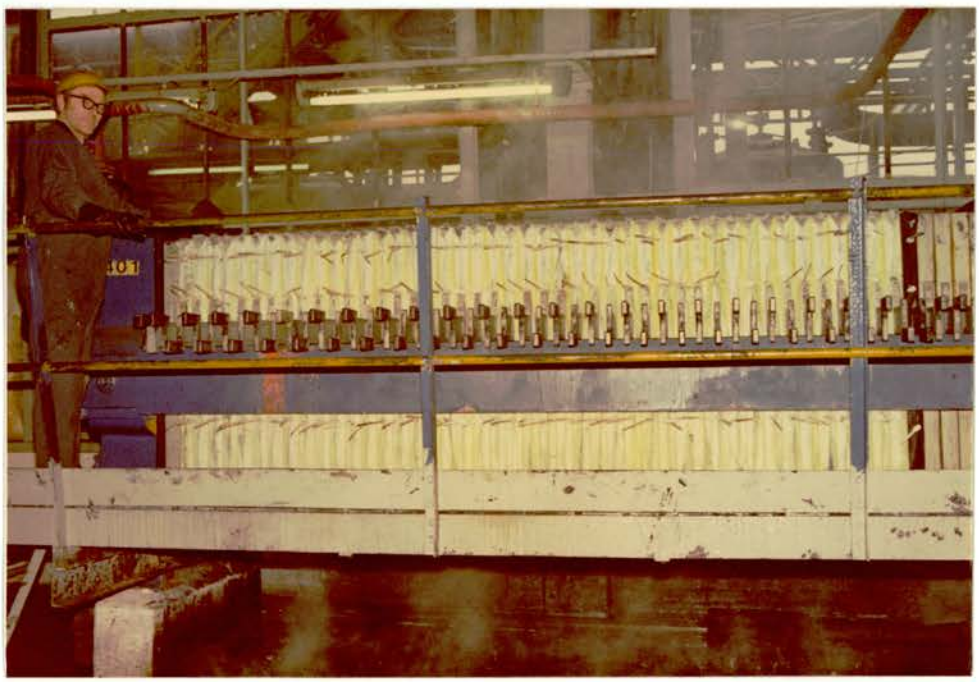


Plate 5

A process operator wearing normal protective clothing on a filter press.

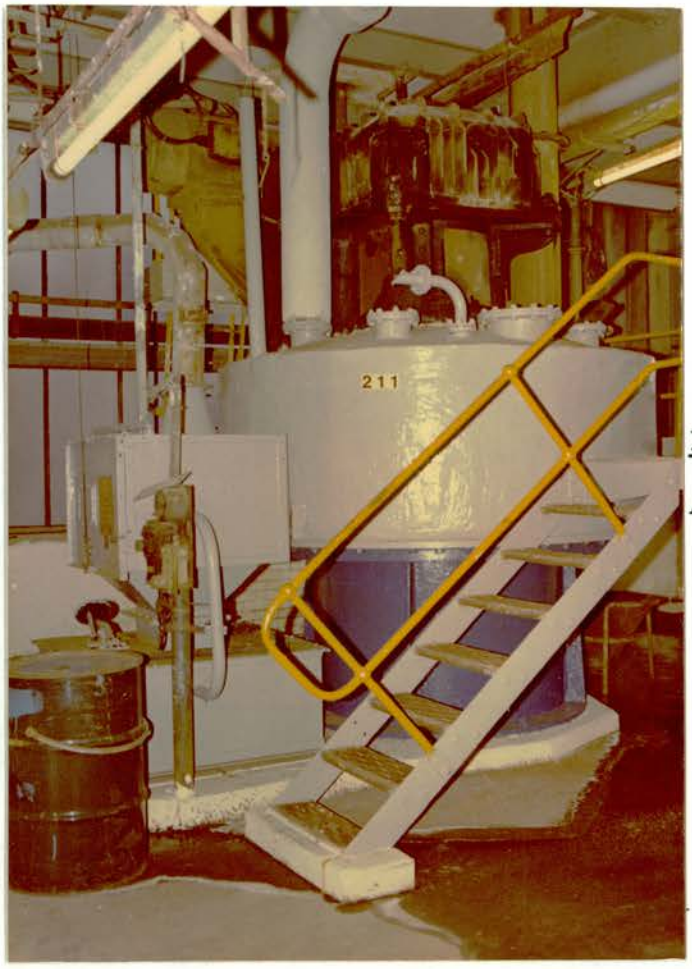


Plate 6

A "Johnson" vacuum dryer.

Plate 7

A process operator operating a vacuum still.

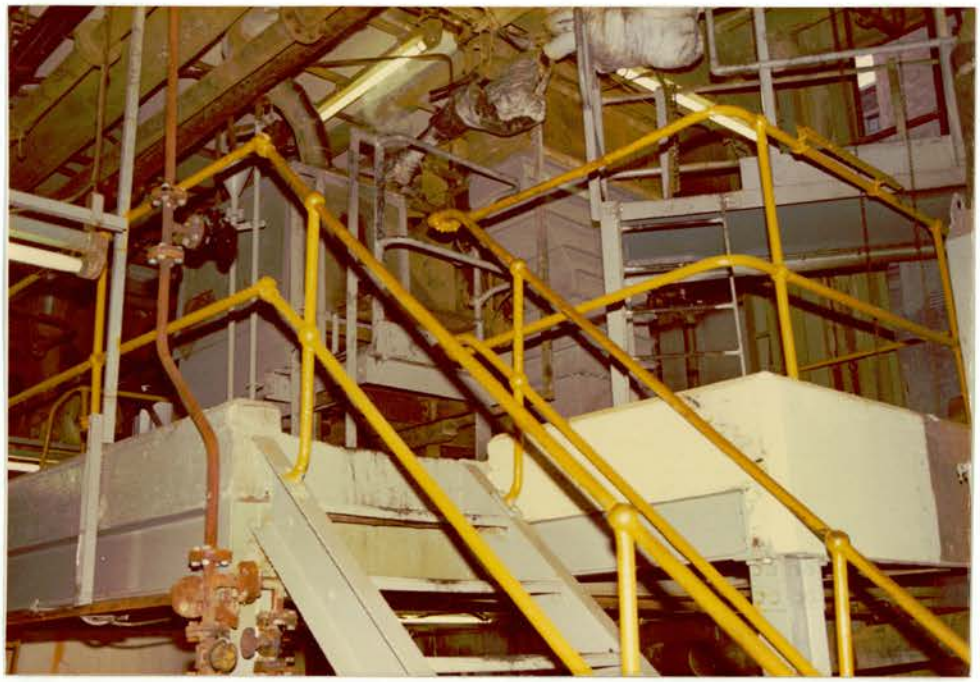


Plate 8

Bandcaster - this cools and flakes the solidified benzanthrone.

Plate 9

Pulverising mill.

3. OUTLINE OF THE PROSPECTIVE INVESTIGATION

I have described both the clinical features of benzanthrone photosensitivity, and also the nature of the problem in the Grangemouth Works of I.C.I. during the manufacturing process.

Objectives of the Investigation

An attempt was undertaken to:

1. Confirm that contact with benzanthrone causes an abnormal photosensitivity state in human skin by testing under controlled conditions.
2. Determine whether the reaction obtained is phototoxic or photoallergic.
3. Study the time course of the reactions.
4. Obtain dose response relationships for the reaction.
5. Determine the wavelengths of ultra violet radiation producing the reaction.
6. Investigate the possible effect of ascorbic acid on the photosensitivity.
7. Investigate some of the mechanisms of the photosensitization reaction level at a cellular level.
8. Investigate the mutagenic potential of benzanthrone.

Chapter IV

DELINEATION OF THE ACTION SPECTRUM IN AFFECTED WORKERS

Including

1. METHODS
   - (a) Xenon Arc Light Source
   - (b) Monochromator
   - (c) Patch Tests and Photopatch Tests
   - (d) Grading and Classification Scale
   - (e) Statistical Analysis.

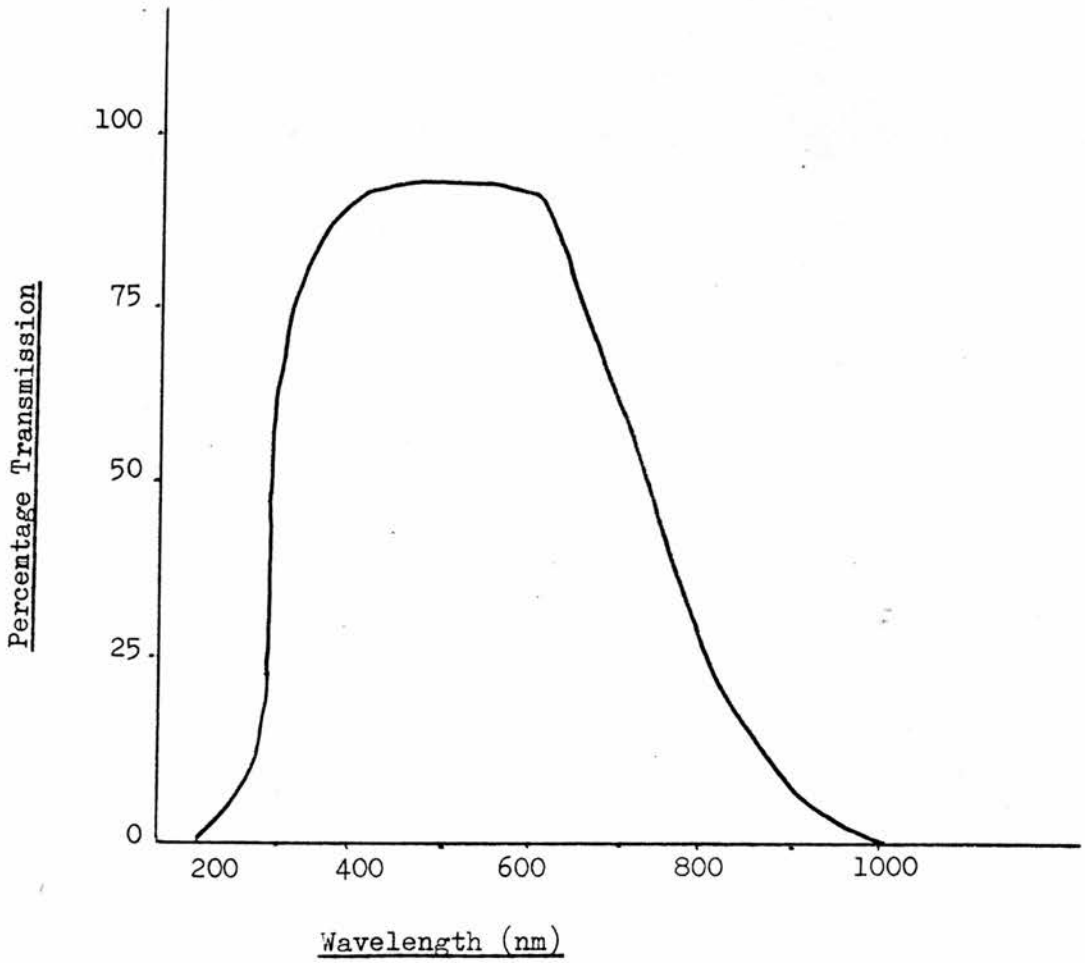
2. THE INVESTIGATION AND RESULTS
   - (a) Experiment to show that for fixed concentrations of benzanthrone the erythema response is linearly related to irradiation time.
   - (b) Results
   - (c) Experiments on the time of exposure to benzanthrone (Protocol 1)
   - (d) Experiments on the time of exposure with varying concentrations of benzanthrone (Protocol 2)
   - (e) Experiments on the time of exposure to benzanthrone (Protocol 3)
   - (f) Results of Protocols 1 - 3.
   - (g) Preliminary studies of wavelength dependence
   - (h) Results
   - (i) Experiments to establish dose response curves at different wavelengths and hence estimate action spectrum
   - (j) Results

1. METHODS

(a) Xenon Arc Light Source

Previous work using a 500 W Xenon light source type XE/D had demonstrated its relatively high UV and infra red emission. The original equipment had been developed by Gardiner (1972) to include an HA 3 heat absorbing filter which reduced this undesirable part of the spectrum. Figure 13 shows the transmission curve of HA 3 infra red absorbing glass.

HA 3 Heat Absorbing Filter

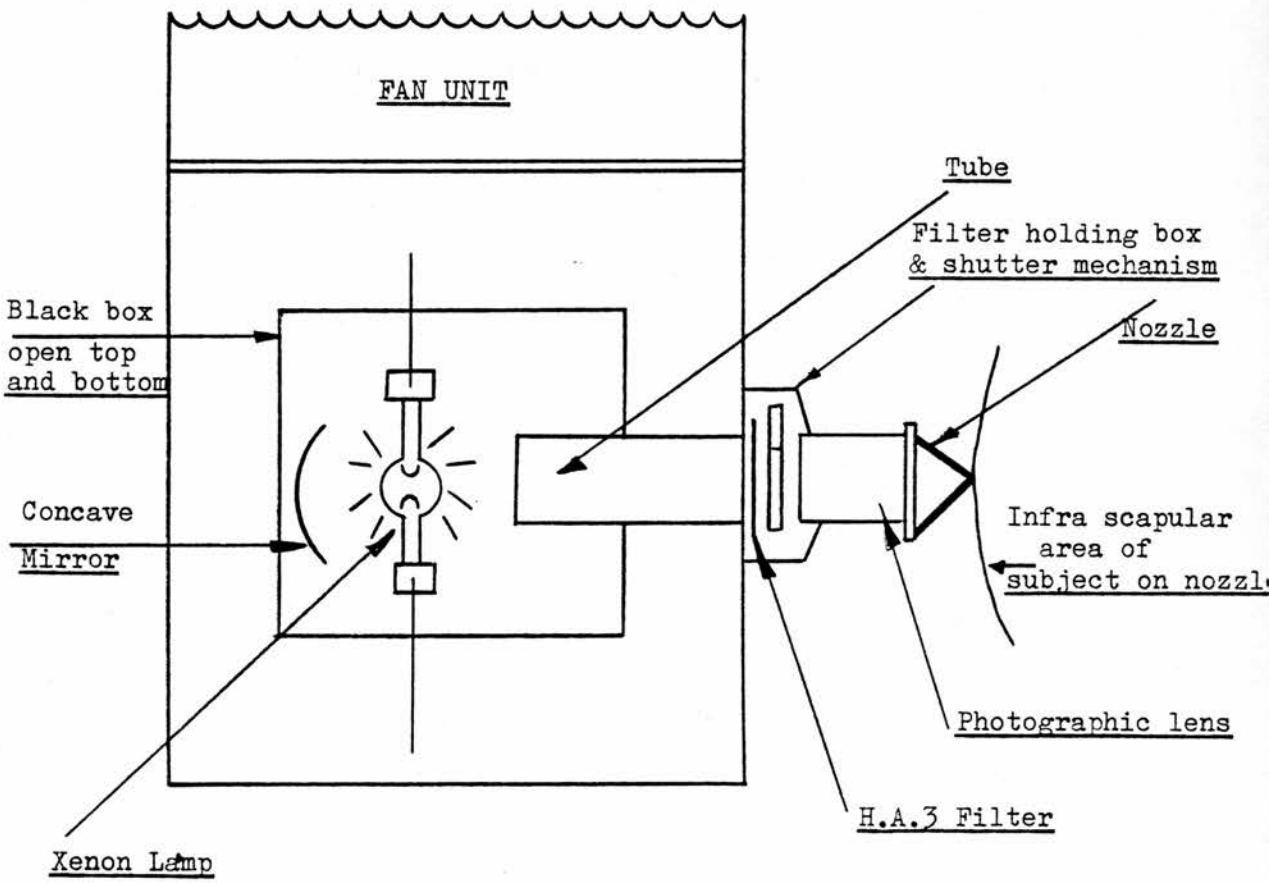


Transmission curve of HA 3 infra red absorbing glass - from manufacturers data.

Fig. 13

A 51 mm diameter tube was inserted between the UV source and the skin surface to increase irradiance.

A short focal length (approx. $1\frac{1}{2}$") photographic lens, the shortest wavelength transmitted by which was 317 nm, was obtained and mounted in the lamp in front of the HA 3 filter. The nozzle being so placed as to be in the focal plane of the lens and giving a small, concentrated, yet uniform energy output. Other cut-off filters can be inserted to facilitate the selection of wavebands. Figure 14 shows the modifications to the 500 W Xenon arc lamp type XE/D.



Diagrammatic representation of 500W Xenon arc lamp Type XE/D with tube, HA3 filter, and photographic lens in position.

Fig. 14

A Hilger-Schwartz thermopile (characteristics shown in Table 2) coupled to a Solartron digital voltmeter was used to measure the energy output at the exit slit in watts per square centimeter. This was done more to check the output over a period of time than to produce data of absolute exposure doses.

TABLE 2

HILGER-SCHWARTZ THERMOPILE

CHARACTERISTICS

Type and Serial No.	FT 4/308
Receiver Dimensions:	2.1 mm Dia.
D.C. Sensitivity:	1.0mV/mW
D.E. Resistance:	52 Ohms
Response Time:	0.04 sec
Window:	Si O_2
Spectral Range:	0.18m - 3.4m or 180 nm - 3400 nm

The irradiance at the skin surface may be calculated from the digital voltmeter reading. The ratio of the filtered to the unfiltered output then gives a measure of the energy above a given wavelength.

Ozone

It was observed during the photopatch testing of the subjects that continuous usage of the Xenon arc lamp produced detectable traces of ozone in the atmosphere. Due to the toxicity of this gas, samples were taken for analysis which revealed that ozone was present at 0.1 ppm, this being the recommended threshold limit value (TLV) as established by the American Conference of Governmental Industrial Hygienists (A.C.G.I.H.).

(b) Monochromator

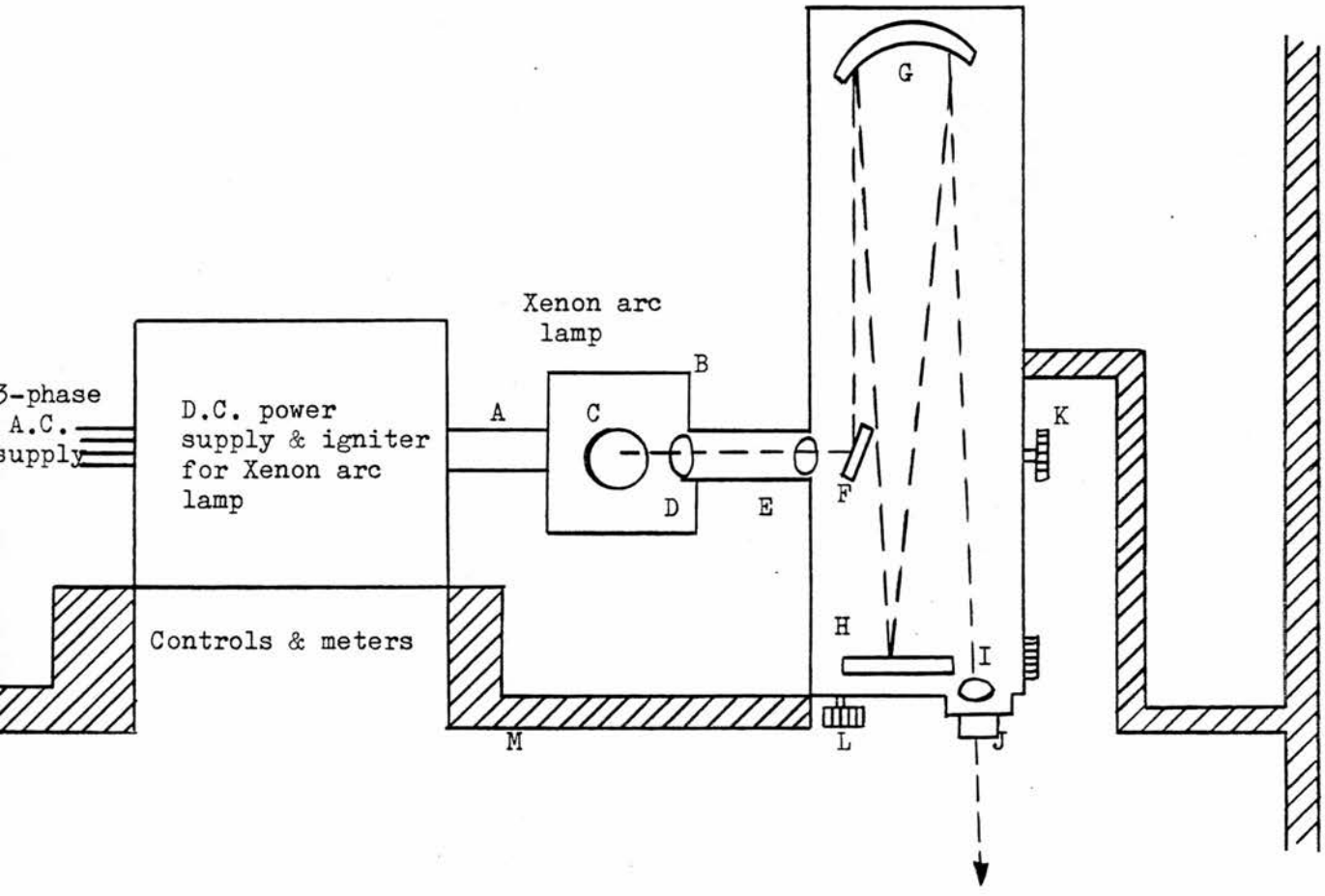
To establish an action spectrum, the skin must be irradiated with narrow wavebands of known energy. This can be done either with the aid of filters, or with a monochromator. With absorption filters, the width of a waveband is wide; with interference filters, somewhat narrower; however, much the narrowest wavebands may be obtained with a monochromator. The actual name monochromator is misleading, as the radiation is only of a more of less narrow waveband, not a single wavelength and it is not necessarily coloured.

MacKenzie & Frain-Bell (1973) described the construction and development of a grating monochromator as used in the Photobiology Unit in the Department of Dermatology, Dundee. Figure 15 shows a schematic layout of their equipment.

The irradiance at the skin surface may be measured using the thermopile and digital voltmeter system as for the xenon arc source, and the exposure doses given, calculated from this measurement.

$$\text{Exposure Dose } (\text{mJcm}^{-2}) = \text{Irradiance } (\text{mWcm}^{-2}) \times \text{Time (sec)}$$

GRATING MONOCHROMATOR



Schematic layout of equipment, as seen from above: A, high tension cables; B, protective lamp housing; C, 1600W Xenon arc lamp; D, Condenser lens; E, entrance slit and lens; F, plane entrance slit mirror; G, concave collimating mirror; H, diffraction grating; I, exit slit and lens; J, shutter, filter holder and nozzle; K, wavelength control; L, slit width controls; M, heat proof, light-tight partition.

Fig. 15

Magnus (1976) described a classical monochromator as

"Briefly the idea is to form a monochromatic image of the entrance aperture and project it at the exit aperture, where it irradiates the target, in this case the skin. The purpose of the condenser is to concentrate the radiant energy of the source on to the entrance slit. The collimator, which follows, forms a parallel beam on to the grating, in which the spectrum is formed. This spectrum most now be focused at the exit slit plane. Lastly, the purpose of the exit slit is to pass only the selected monochromatic image of the entrance slit and its plate is a light stop for other wavelengths. Diffraction by a grating results in the formation of a series of spectra by wave interference".

(c) Patch and Photopatch Tests

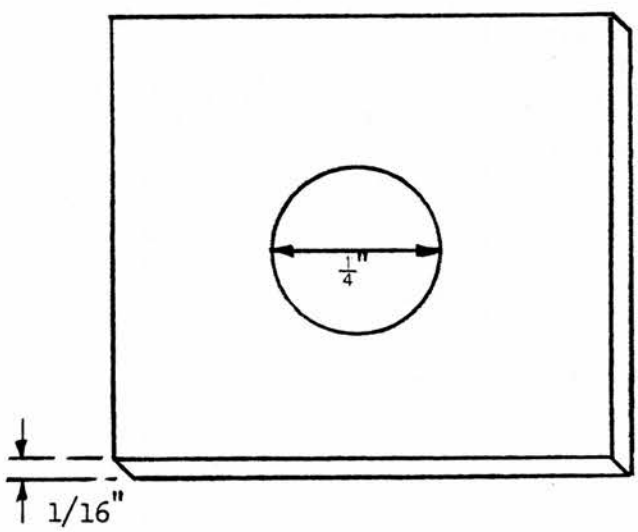
Throughout the entire investigation aluminium backed patches (Al-test patches, Imeco Astra Agency AB, Södertalje, Sweden) were used. These were kept in place with adhesive tape (polyvent adhesive tape, manufactured by Kendall Hospital Products, Boston, Massachusetts, U.S.A.).

The actual patch and photopatch test technique used is described in the introduction to this thesis, though it should be added that there are certain disadvantages to the Al-test units. Reactions to the polythene covering on the aluminium foil occasionally occur, causing erythema and slight oedema around the filter paper discs. Usually only some units, possible a strip, are affected, but occasionally all the tests are involved to a greater or lesser extent. The redness is usually most pronounced at two days. This reaction is a distinct disadvantage because by increasing the irritability of the test area false positive reactions become more likely and add to the difficulty of interpreting test results. The cause of the reaction is unknown but Fregert (1972) suggested that excess heat or ozone may oxidise one patch of polythene during its fixation to the aluminium. Polythene aged by ultra violet light and oxygen is changed in the same way.

The following device (Figure 16) was employed to ensure that each Al-test patch contained a standard amount of benzanthrone. A hole $\frac{1}{4}$" in diameter, and similar in diameter to the Al-test filter paper disc, was cut in a perspex square of 1/16" thickness. The perspex template was then placed over the Al-test disc such that the hole and disc co-incided, the disc being 1/16" beneath the

surface of the perspex. Benzanthrone, diluted in PMF*, was then applied to the template with a spatula filling the $\frac{1}{4}$" diameter hole.

* PMF = Paraffin Molle Flavum.



Perspex square

Fig. 16

(d) Photopatch Test Grading and Classification Scale

A grading and classification scale was evolved to facilitate interpretation of the photopatch results - Table 3.

GRADING AND CLASSIFICATION SCALE

Grade	Classification
0	No erythema
0.5	Doubtful erythema
1	Faint but definite erythema
2	Marked erythema
3	Marked erythema with minimal oedema
4	Marked erythema with marked oedema
5	Blistering
6	Pigmentation

Table 3

(e) Regression Analysis

Least squares regression analysis is a statistical technique for empirically estimating the form of a relationship between variables. In the simplest case, in which only two variables are being considered, the analysis reveals (1) whether or not there is a relationship between the variables, (2) the most probable form of the relationship (i.e. whether the variables exhibit a linear relationship, or a quadratic relationship etc.) and (3) the most probable estimates of the parameters which define the relationship.

With the assistance of a computer-based regression analysis package the technique may be extended to the identification of a relationship between a group of "predictor" variables (or "explanatory" variables) and a "dependent" or "response" variable. From a given set of predictor variables the technique selects those which have the most significant effect on the response variable and estimates the parameters of the relationship identified.

The data required in order to undertake a regression analysis consists of several "observations" of the variables involved. Each observation will be a summary of the conditions for a particular experiment, in the form of numeric data recorded for each predictor variable, together with the quantification of the "response" which those particular conditions induced. If, for example, the relationship of interest is that between a response Y and two predictor variables X and T, then several small experiments would need to be undertaken with the experimenter manipulating the variables X and T to give a range of different conditions. For each experiment the values of the variables X and T would be recorded together with the induced response Y. The set of three values (one for each variable) makes up a single

observation, and each repeat of the experiment would produce a new observation.

In addition to the predictor variables which the experimenter has manipulated, theoretical considerations may suggest that some transformation of one (or more) of these variables could be a meaningful predictor. Thus, in attempting to find a relationship between the response Y and predictors X and T, it may be appropriate to form the variables T^2 and LOG_e (X) as possible predictor variables, or even to form the interaction variable XT (i.e. X multiplied by T).

The results of a regression analysis will be an equation which defines the most meaningful relationship between the response variable and the predictor variables. This will take the form (for example)

$$Y = a + b_1X + b_2T + b_3T^2$$

The analysis reveals which predictor variables are most definitely related to the response variable, and leaves other variables out of the equation. (The selection procedure is based on a pre-defined level of significance for the inclusion of a variable: those variables which fail to meet this significance level are omitted from the equation). The analysis also gives estimates of the coefficients a, b_1, b_2 etc. using the least squares criterion. If the equation is linear then the coefficient for a variable is the estimated effect on the response of a unit increase in that variable, assuming that other conditions remain constant.

The fitted regression equation may be used to estimate the average or predicted response to any given set of experimental conditions.

2. THE INVESTIGATION AND RESULTS

(a) Initial Investigation

Subject (1) SAW (self) was patch tested on the infra scapular zone with 2% benzanthrone in PMF for 24 hours and then exposed to the 500W Xenon arc lamp for an arbitrary time of 1, 5, 10 and 15 minutes. The reaction was graded as either a positive or negative erythema response.

Subsequently 29 other subjects were photopatch tested in a similar manner with concentrations of benzanthrone of ¼%, 1% and 2% diluted in PMF, again the reactions being graded as either positive or negative erythema responses.

The original test (SAW) showed an erythema response with exposures of 10 and 15 minutes. The subsequent work was therefore carried out mainly with exposure times of 3, 5, 6, 7 and 10 minutes.

The majority of subjects showed positive results with 5, 6 and 7 minute exposures - see table 4 in which (+) represents a positive photopatch test and (-) represents a negative photopatch test. An empty box in table 4 indicates that exposures had not been made.

(b) Results

Table 4 summarises the results of the initial photopatch studies on the 30 subjects. In all, 90 reaction observations were obtained for varying concentrations of benzanthrone and irradiation times. (Two of these observations were subsequently ignored as they were the only observations with 15 minutes of irradiation). For this analysis, the erythema response which is recorded as '+' and '-' in Table 4 was coded as '1' and '0'. The regression analysis fitted the following equation -

$$\text{Erythema response} = (0.175 - 0.0169 \times \text{benzanthrone conc})^2 \times \text{irradiation time.}$$

The equation is an empirical estimate of the relationship between the 'average' erythema response and the two predictor variables. Since the erythema response was recorded as simply '+' or '-' (i.e. '1' or '0') the 'average' response can also be interpreted as the response rate i.e. the proportion of subjects showing a positive response. The equation indicates that for fixed concentrations of benzanthrone the erythema response is linearly related to the irradiation time, with the response rate increasing as the irradiation time is increased. The fitted equation also suggests that for fixed irradiation times the response has a quadratic relationship with benzanthrone concentration, with the response rate <u>decreasing</u> as the concentration increases. *

Table 5 gives the actual and predicted response rates for the experimental conditions investigated.

* Note however the different result obtained from the second, more detailed investigation - See page 95.

INITIAL PHOTOPATCH STUDIES

DATE	SUBJECT	CONC BENZANTHRONE IN P.M.F.			PATCH TIME ON SKIN	EXPOSURE TIME WITH ERYTHEMA RESPONSE MINUTES							
		2%	1%	½%		0	1	3	5	6	7	10	15
19.5.77.	SAW (1)	2%			24 hours		-		-			+	+
25.5.77.	AC) (2)	2%			"				+		+	+	+
1.7.77.	AC)			½%	"				+				
1.7.77.	AC)		1%		"					+			
26.5.77.	MM (3)	2%			"				+			+	
26.5.77.	CF) (4)	2%			"				+			+	
30.6.77.	CF)		1%		"					+			
30.6.77.	CF)			½%	"				+				
26.5.77.	RR (5)	2%			"				+		-		
27.5.77.	JS (6)	2%			"				+		-		
31.5.77.	FL) (7)	2%			"			+	+				
5.7.77.	FL)			½%	"						+		
5.7.77.	FL)		1%		"					+			
31.5.77.	DG) (8)	2%			"			+	+		+		
27.6.77.	DG)			½%	"				-				
27.6.77.	DG)		1%		"					+			
1.6.77.	KJ (9)	2%			"			-	-				
10.6.77.	SC (10)	2%			"			-	-				
10.6.77.	WA)(11)	2%			"			-	-		+		
30.6.77.	WA)		1%		"					+			
30.6.77.	WA)			½%	"				+				
10.6.77.	TW)(12)	2%			"			-	-		+		
30.6.77.	TW)			½%	"				-				
30.6.77.	TW)		1%		"					+			
10.6.77.	JA)(13)	2%			"			-	-		+		
30.6.77.	JA)			½%	"				+				
30.6.77.	JA)		1%		"					+			
10.6.77.	AH)(14)	2%			"			-	-		+		
30.6.77.	AH)			½%	"				+				
30.6.77.	AH)		1%		"					+			
16.6.77.	JA (15)	2%			"				-		-		
16.6.77.	WM (16)	2%			"				+		+		
16.6.77.	RR (17)	2%			"				+		+		
16.6.77.	MK (18)	2%			"				-		+		
16.6.77.	HM (19)	2%			"				-		-		
23.6.77.	RH (20)	2%			"						+	+	
23.6.77.	JM (21)	2%			"						+	+	
23.6.77.	TH (22)	2%			"						-	+	
23.6.77.	JC (23)	2%			"						+	+	
23.6.77.	JJ (24)	2%			"						+	+	
1.7.77.	PL)(25)	2%			"						+		
1.7.77.	PL)		1%		"					+			
1.7.77.	PL)			½%	"				+				
6.7.77.	RR)(26)	2%			"						+		
6.7.77.	RR)		1%		"					+			
6.7.77.	RR)			½%	"				+				
6.7.77.	BM)(27)	2%			"						+		
6.7.77.	BM)		1%		"					+			
6.7.77.	BM			½%	"				+				
6.7.77.	AK)(28)	2%			"						+		
6.7.77.	AK)		1%		"					+			
6.7.77.	AK)			½%	"				+				
6.7.77.	IS)(29)	2%			"						+		
6.7.77.	IS)		1%		"					+			
6.7.77.	IS)			½%	"				+				
6.7.77.	PB)(30)	2%			"						+		
6.7.77.	PB)		1%		"					+			
6.7.77.	PB)			½%	"				+				

Table 4

ACTUAL AND PREDICTED RESPONSE RATES
FROM 3-10 MINUTES

Conc Benzanthrone \ Light Time (mins)		3	5	6	7	10
¼%	Act		84.6% (13)		100% (1)	
	Pred		87.1%		100% +	
1%	Act			100% (14)		
	Pred			95%		
2%	Act	25% (8)	47.4% (19)		83.3% (24)	100% (9)
	Pred	32.3%	53.8%		75.3%	100% +

Figures in parentheses show number of observations.

Table 5.

Protocols 1 - 3 evolved from these initial experiments which showed that, for fixed concentrations of benzanthrone, the erythema response was linearly related to the irradiation time, with the response rate increasing as the irradiation time was increased.

(c) Protocol 1

The Effect of Duration of Contact with Benzanthrone on Skin Photosensitization

Six subjects were patch tested with 2% benzanthrone in PMF for periods of 1, 2, 4, 8, 24 and 48 hours. Each patch was subsequently irradiated for 7 minutes (this being the time selected from the initial investigation which had the largest number of observations), and any reactions classified and graded at 1, 2, 4, 8, 16, 24, 32, 48 and 96 hours, 1 week and 2 weeks post exposure to light. These results are shown in Table 6 and summarised on page 95.

PROTOCOL 1

Patch time on skin	Xenon arc lamp exposures	Subject	Post reaction exposures to light Grading classification										
			1hr	2	4	8	16	24	32	48	96	1week	2week
1 Hour	7 mins.	RR	1	1	1	0.5	0.5	0	0	0	0	0	0
		PB	0	0	0	0.5	0	0	0	0	0	0	0
		MM	0.5	1	1	1	0.5	0	0	0	0	0	0
		JS	1	1	2	1	1	1	1	0.5	0	0	0
		SAW	1	1	1	1	0.5	0.5	0.5	0	0	0	0
		CF	2	2	2	1	1	1	0.5	0.5	0	0	0
2 Hour	7 mins.	RR	1	1	1	0.5	0.5	0.5	0.5	0	0	0	0
		PB	1	1	1	1	0.5	0	0	0	0	0	0
		MM	1	2	2	2	0.5	0.5	0	0	0	0	0
		JS	2	2	2	2	2	2	2	1	0	0	0
		SAW	2	2	2	2	1	1	0.5	0	0	0	0
		CF	2	2	2	1	1	1	0.5	0.5	0	0	0
4 Hour	7 mins.	RR	2	2	1	0.5	0.5	0.5	0.5	0	0	0	0
		PB	0	0	1	2	1	1	1	0	0	0	0
		MM	0.5	1	1	1	1	0.5	0	0	0	0	0
		JS	2	1	1	1	1	1	1	0	0	0	0
		SAW	2	2	2	2	1	1	0.5	0.5	0	0	0
		CF	2	2	1	0.5	0.5	0.5	0	0	0	0	0
8 Hour	7 mins.	RR	2	2	2	1	1	1	1	0.5	0	0	0
		PB	0	0	1	1	0.5	0	0	0	0	0	0
		MM	0.5	0.5	1	1	0.5	0.5	0.5	0	0	0	0
		JS	2	2	2	2	2	2	1	0	0	0	0
		SAW	2	2	2	2	2	1	1	0.5	0	0	0
		CF	2	2	2	1	1	1	1	0	0	0	0
24 Hour	7 mins.	RR	1	1	1	2	2	2	1	2	0	0	0
		PB	0	2	1	1	1	0	0	0	0	0	0
		MM	2	2	2	2	1	1	0	0	0	0	0
		JS	0.5	0.5	0.5	1	2	2	2	0	0	0	0
		SAW	1	1	1	1	1	1	0.5	0	0	0	0
		CF	2	2	2	1	0	0	0	0	0	0	0
48 Hour	7 mins.	RR	2	2	2	2	1	1	1	0	0	0	0
		PB	0.5	0.5	0.5	0	0	0	0	0	0	0	0
		MM	1	1	1	1	1	1	1	0.5	0.5	0	0
		JS	2	2	2	2	2	1	1	1	0	0	0
		SAW	2	2	2	2	2	1	1	0.5	0.5	0	0
		CF	2	2	2	2	2	2	1	0	0	0	0

Table 6

(d) Protocol 2

The Effect of Varying Concentrations of Benzanthrone

A further group of subjects was patch tested with PMF alone (0% benzanthrone), $\frac{1}{4}$% benzanthrone, 1% benzanthrone and 2% benzanthrone in PMF over a 24 hour period. Each patch was subsequently irradiated for 7 minutes and any reactions were graded and classified at 1, 2, 4, 8, 16, 24, 32, 48 and 96 hours, 1 week and 2 weeks post exposure to light. These results are shown in Table 7 and summarised on page 95.

PROTOCOL 2

Patch time on skin	Xenon arc lamp exposure	Conc Benz	Subject	Post reaction exposure to light Grading classification										
				1hr	2	4	8	16	24	32	48	96	1week	2week
24 hr	7 mins	0%	PB	1	1	0	0	0	0	0	0	0	0	0
"	"	0%	PL	0	0	0	0	0	0	0	0	0	0	0
"	"	0%	MK	0.5	0.5	0.5	0.5	1	1	1	1	0.5	0	0
"	"	0%	RR	0	0	0	0	0	0	0	0	0	0	0
"	"	0%	JA	0	0	0	0	0	0	0	0	0	0	0
"	"	0%	RS	1	1	1	0	0	0	0	0	0	0	0
24 hr	7 mins	¼%	PB	1	1	1	1	1	0	0	0	0	0	0
"	"	¼%	PL	2	2	3	1	1	1	1	1	0	0	0
"	"	¼%	MK	0	0	0	0	2	3	3	2	1	0	0
"	"	¼%	RR	0	0.5	0	0	0	0	0	0	0	0	0
"	"	¼%	JA	0.5	0.5	0.5	0	0	0	0	0	0	0	0
"	"	¼%	RS	1	2	2	1	1	1	1	0.5	0	0	0
24 hr	7 mins	1%	PB	1	1	1	1	1	0.5	0.5	0	0	0	0
"	"	1%	PL	2	2	3	2	2	1	1	1	1	0	0
"	"	1%	MK	2	2	2	2	1	1	1	1	1	0.5	0
"	"	1%	RR	0	1	0	0	0	0	0	0	0	0	0
"	"	1%	JA	1	1	1	0.5	0.5	0	0	0	0	0	0
"	"	1%	RS	1	2	2	1	1	1	1	0.5	0	0	0
24 hr	7 mins	2%	PB	1	1	1	1	1	0.5	0.5	0.5	0	0	0
"	"	2%	PL	2	2	3	2	2	1	1	1	1	1	0.5
"	"	2%	MK	2	2	2	2	1	0.5	1	0.5	0.5	0	0
"	"	2%	RR	0	2	0	0	0	0	0	0	0	0	0
"	"	2%	JA	2	2	2	0.5	0.5	0	0	0	0	0	0
"	"	2%	RS	1	2	2	1	1	1	1	0.5	0	0	0

Table 7

(e) Protocol 3

Supplementary Study of the Time Course for Benzanthrone Induced Photosensitivity Reaction in Skin

A further group of 6 subjects was patch tested with PMF alone (0% benzanthrone) and 2% benzanthrone in PMF over a 24 hour period. Each patch was then subsequently irradiated for 7 minutes and any reactions graded and classified at 1, 2, 4, 8, 16, 24, 32, 48 and 96 hours and 1 week and 2 weeks post exposure to light. These results are shown in Table 8 and summarised on page 95.

PROTOCOL 3

Patch time on skin	Xenon arc lamp exposure	Conc Benz	Subject	Post reaction exposure to light Grading classification										
				1hr	2	4	8	16	24	32	48	96	1week	2week
24 hr	7 mins	0%	FL	0	0	0	0	0	0	0	0	0	0	0
"	"	0%	RR	0	0	0	0	0	0	0	0	0	0	0
"	"	0%	IS	0	0	0	0	0	0	0	0	0	0	0
"	"	0%	DG	0	0	0	0	0	0	0	0	0	0	0
"	"	0%	AK	0	0	0	0	0	0	0	0	0	0	0
"	"	0%	BM	0	0	0	0	0	0	0	0	0	0	0
24 hr	7 mins	2%	FL	1	1	1	2	1	1	1	0	0	0	0
"	"	2%	RR	0	0	0	2	2	1	1	1	1	0	0
"	"	2%	IS	1	0.5	0.5	0.5	0.5	0	0	0	0	0	0
"	"	2%	DG	0.5	1	2	1	1	0.5	0.5	0	0	0	0
"	"	2%	AK	0	0	0	0	0	1	2	2	2	0	0
"	"	2%	BM	0	0	0	2	2	2	2	0.5	0	0	0

Table 8

(f) Results

The results obtained in Protocols 1, 2 and 3 were combined to assess the dependence of the grading classification of redness on (a) the patch time, (b) the delay time, and (c) the benzanthrone concentration. (The irradiation time was constant throughout the trials). The regression analysis fitted the following equation

$$\text{Redness grading} = 0.13 + 1.33\,(\text{concentration}) - 0.37\,(\text{concentration})^2 + 0.038 \log(\text{patch time}) + 0.079 \log(\text{delay time}) - 0.027\left[\log(\text{delay time})\right]^2 - 0.098\,(\text{concentration})\left[\log(\text{delay time})\right]$$

The residual standard deviation of the equation is 0.59

This equation can be interpreted as follows :-

(i) if the concentration of benzanthrone and the patch time are held constant, the average redness grading decreases as the (logarithm of the) delay time increases, as one might expect (see Graph 1).

(ii) if the patch time and the delay time are held constant, the average redness grading has a quadratic relationship with the benzanthrone concentration, with the redness grading having a maximum value for concentrations between 1% and 2% depending on the delay time. (See graph 2.) *

(iii) if the concentration of benzanthrone and the delay time are held constant, the average redness grading has a quadratic relationship with the (logarithm of the) patch time, with the average grading increasing as the (logarithm of the) patch time increases (see Graph 3).

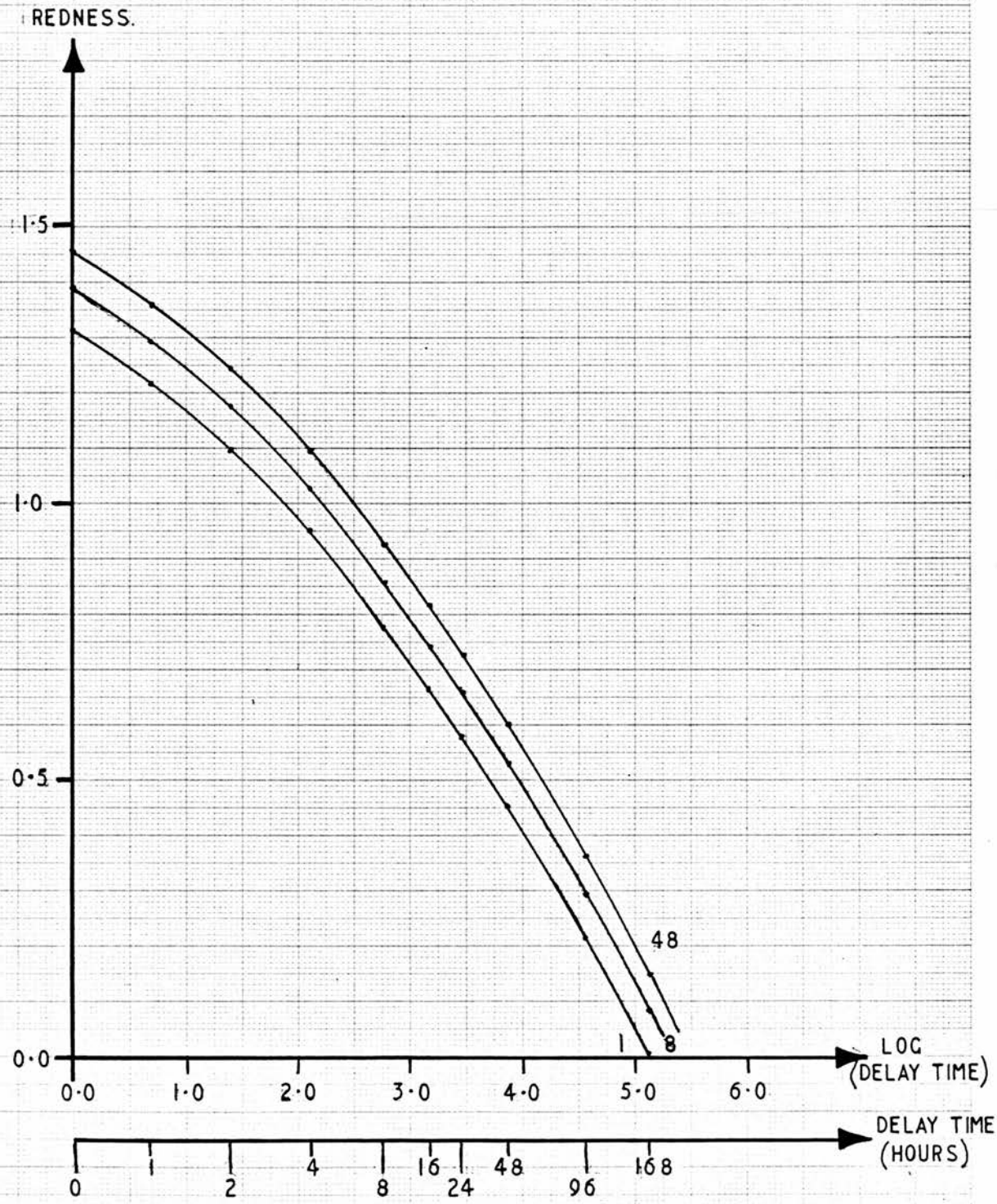
Table 9 displays the observed value along with the value predicted by the above regression equation, for each of the conbinations of patch time, delay time and concentrations.

* The result of this investigation indicates a different form of quadratic relationship between the reaction effect and the benzanthrone concentration to that of the initial investigation. However, the former investigation is based on a very sparse experimental design, and this aspect of the results should perhaps be overlooked.

Note: The fitted equation has not been forced to pass through the point (concentration = 0; redness grading = 0) since, in practice, this does not always occur.

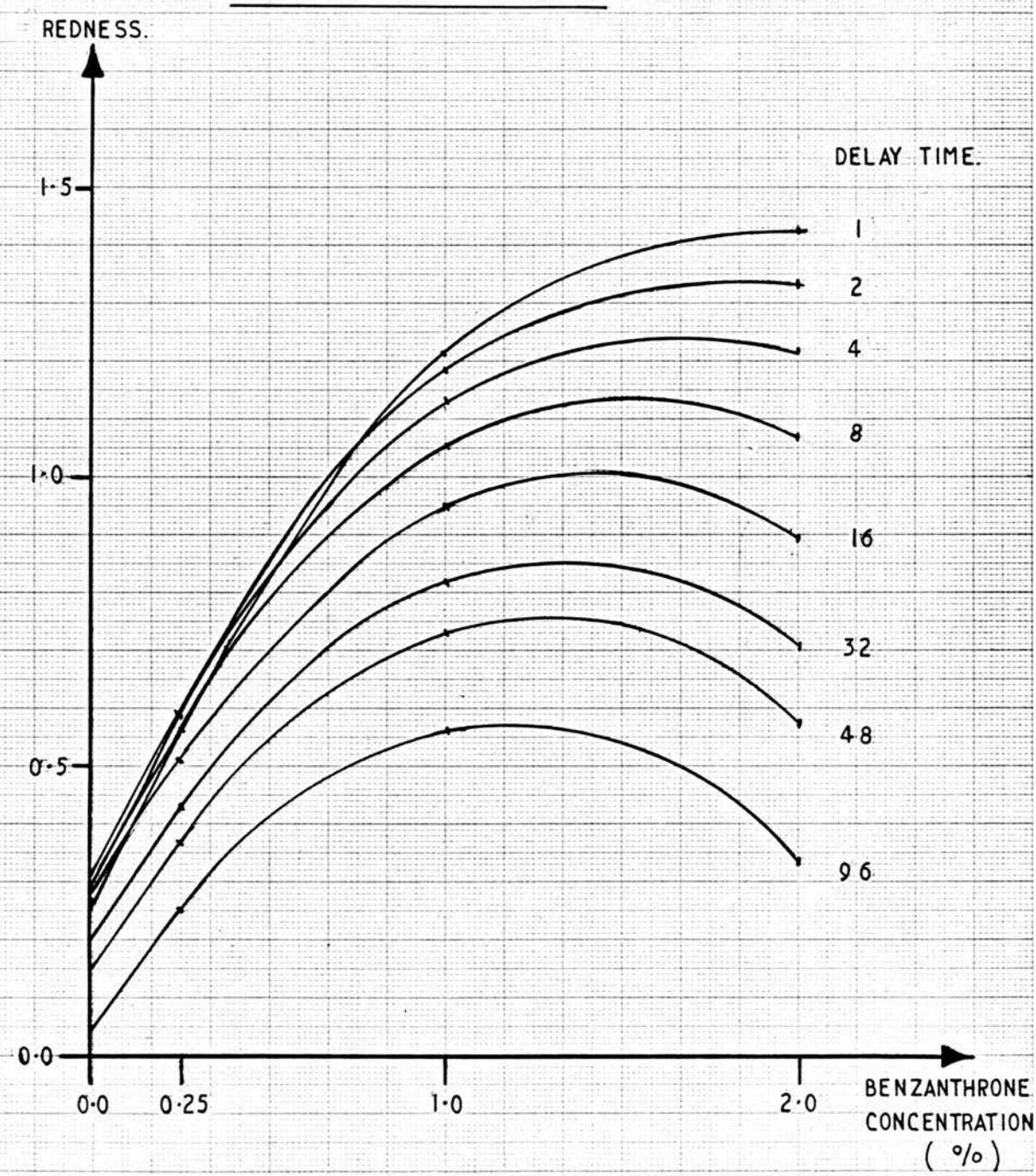
GRAPH 1

BENZANTHRONE CONCENTRATION 2 %



GRAPH II

PATCH TIME- 24 HOURS.



GRAPH III

BENZANTHRONE CONCENTRATION 2 %

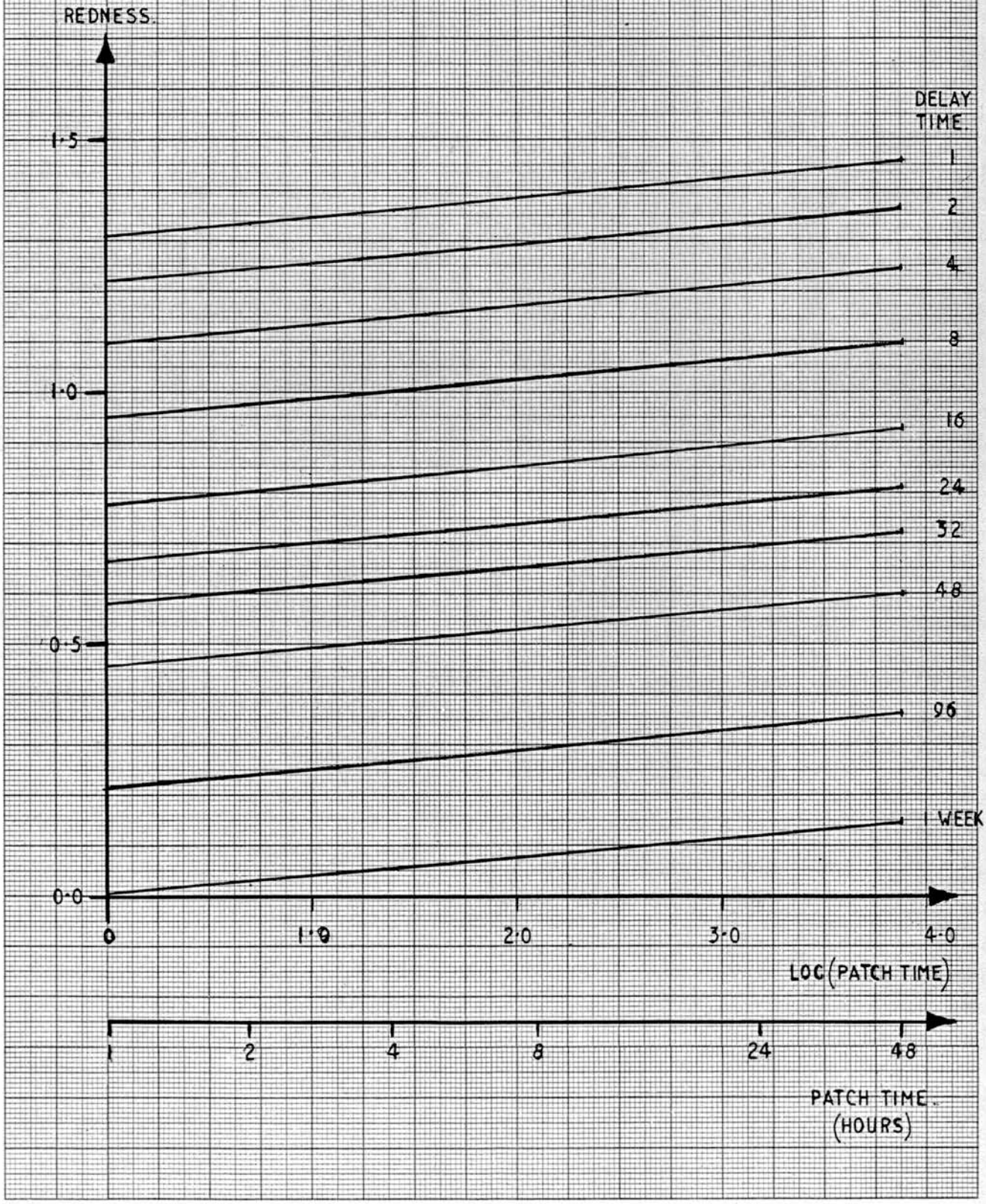


Table 9

Concentration	Patch Time	DELAY TIME											
		1 hr		2 hr		4 hr		8 hr		16 hr		24 hr	
		Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred
2%	1 hr	0.92	1.3	1.00	1.2	1.16	1.1	0.83	1.0	0.58	0.8	0.42	0.7
	2 hr	1.50	1.3	1.67	1.2	1.67	1.1	1.42	1.0	0.92	0.8	0.83	0.7
	4 hr	1.42	1.4	1.33	1.3	1.17	1.2	1.17	1.0	0.83	0.8	0.75	0.7
	8 hr	1.42	1.4	1.42	1.3	1.67	1.2	1.33	1.0	1.17	0.9	0.92	0.7
0%	24 hr	0.21	0.3	0.21	0.3	0.12	0.3	0.04	0.3	0.08	0.3	0.08	0.2
½%	24 hr	0.75	0.6	1.00	0.6	1.08	0.6	0.50	0.6	0.83	0.5	0.83	0.5
1%	24 hr	1.17	1.2	1.50	1.2	1.50	1.1	1.08	1.1	0.92	1.0	0.58	0.9
2%	24 hr	0.94	1.4	1.22	1.3	1.17	1.2	1.22	1.1	1.06	0.9	0.81	0.8
2%	48 hr	1.58	1.5	1.58	1.4	1.58	1.2	1.50	1.1	1.33	0.9	1.00	0.8

Concentration	Patch Time	DELAY TIME											
		32 hr		48 hr		96 hr		1 wk		2wks			
		Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred		
2%	1 hr	0.33	0.6	0.17	0.5	0	0.2	0	0	0	0		
	2 hr	0.58	0.6	0.25	0.5	0	0.2	0	0	0	0		
	4 hr	0.50	0.6	0.08	0.5	0	0.3	0	0	0	0		
	8 hr	0.75	0.7	0.17	0.5	0	0.3	0	0.1	0	0		
0%	24 hr	0.08	0.2	0.08	0.1	0.04	0	0	0	0	0		
½%	24 hr	0.83	0.4	0.58	0.4	0.17	0.2	0	0.1	0	0		
1%	24 hr	0.58	0.8	0.42	0.7	0.33	0.6	0.08	0.4	0	0.2		
2%	24 hr	0.75	0.7	0.44	0.6	0.25	0.3	0.06	0.1	0.3	0		
2%	48 hr	0.83	0.7	0.17	0.6	0.17	0.4	0	0.1	0	0		

(g) Preliminary Studies of Wavelength Dependence

Taking into consideration the results of the analysis of Protocols 1 - 3, a concentration of 2% benzanthrone in PMF and a contact time of 2 hours was selected for the subsequent investigations involving the Xenon arc lamp in the initial elucidation studies of the action spectrum.

Method

A group of 6 subjects was patch tested with 2% benzanthrone in PMF for 2 hours. The patches were then irradiated using Schott "cut off" filters of WG 350, GG 400 and OG 500 for 1, 3, 5, 7 and 10 minutes and the results graded and classified at 2 hour and 4 hour post exposure. These results are shown in Table 10 and summarised on page 102.

Subject	Conc Benz	Filter	Patch time on skin	Patch exposure time to light						Patch exposure grading reaction											
				Minutes						2 Hours						4 Hours					
				0	1	3	5	7	10	0	1	3	5	7	10	0	1	3	5	7	10
CF	2%	500nm	2 hours	"	"	"	"	"	"	0	0	0	0	0	0	0	0	0	0	0	0
JS	"	"	"	"	"	"	"	"	"	0	0	0	0	0	0	0	0	0	0	0	0
SAW	"	"	"	"	"	"	"	"	"	0	0	0	0	0	0	0	0	0	0	0	0
RR	"	"	"	"	"	"	"	"	"	0	0	0	0	0.5	1	0	0	0	0	0	0
MM	"	"	"	"	"	"	"	"	"	0	0	0	0	0	0	0	0	0	0	0	0
MC	"	"	"	"	"	"	"	"	"	0	0	0	0	0	0	0	0	0	0	0	0
CF	2%	400nm	2 hours	"	"	"	"	"	"	0	0	0	0	0	0	0	0	0	0	0	0
JS	"	"	"	"	"	"	"	"	"	0	0	0	0	0	0	0	0	0	0	0	0
SAW	"	"	"	"	"	"	"	"	"	0	0	0	0	0	0	0	0	0	0	0	0
RR	"	"	"	"	"	"	"	"	"	0	0	0	0	0.5	1	0	0	0	0	0	0
MM	"	"	"	"	"	"	"	"	"	0	0	0	0	0	0	0	0	0	0	0	0
MC	"	"	"	"	"	"	"	"	"	0	0	0	2	2	2	0	0	0	2	2	2
CF	2%	350nm	2 hours	"	"	"	"	"	"	0	1	1	1	1	1	0	0	1	1	1	1
JS	"	"	"	"	"	"	"	"	"	0	0	0.5	1	1	1	0	0	0	1	1	1
SAW	"	"	"	"	"	"	"	"	"	0	0	0	0	0	0	0	0	0	1	1	1
RR	"	"	"	"	"	"	"	"	"	0	0	0	0	1	1	0	0	0	0	1	1
MM	"	"	"	"	"	"	"	"	"	0	0	0	1	1	1	0	0	0	0	1	1
MC	"	"	"	"	"	"	"	"	"	0	0	2	2	2	2	0	0	2	2	2	2

Table 10

(h) Results

The results of this experiment are shown in Table 10. They were analysed using regression analysis to assess the effect of the filters on the average redness grading. The following equation was fitted :-

Redness grading = 1.969 x irradiation time

$$-0.00833 \times \text{filter wavelength} \times \text{irradiation time}$$

$$+ 0.00000882 \times (\text{filter wavelength})^2 \times \text{irradiation time}$$

The equation confirms that the redness grading is linearly related to the irradiation time (when the filter wavelength is held constant) : see Graph 4. When irradiation time is held constant the redness grading has a quadratic relationship with the filter wavelength, with average redness grading decreasing as the wavelength increases. The analysis failed to reveal any relationship between the redness grading and the delay time in this experiment, though this cannot be considered a serious contradiction of previous results since only two different delay times were used, and these were of a similar order.

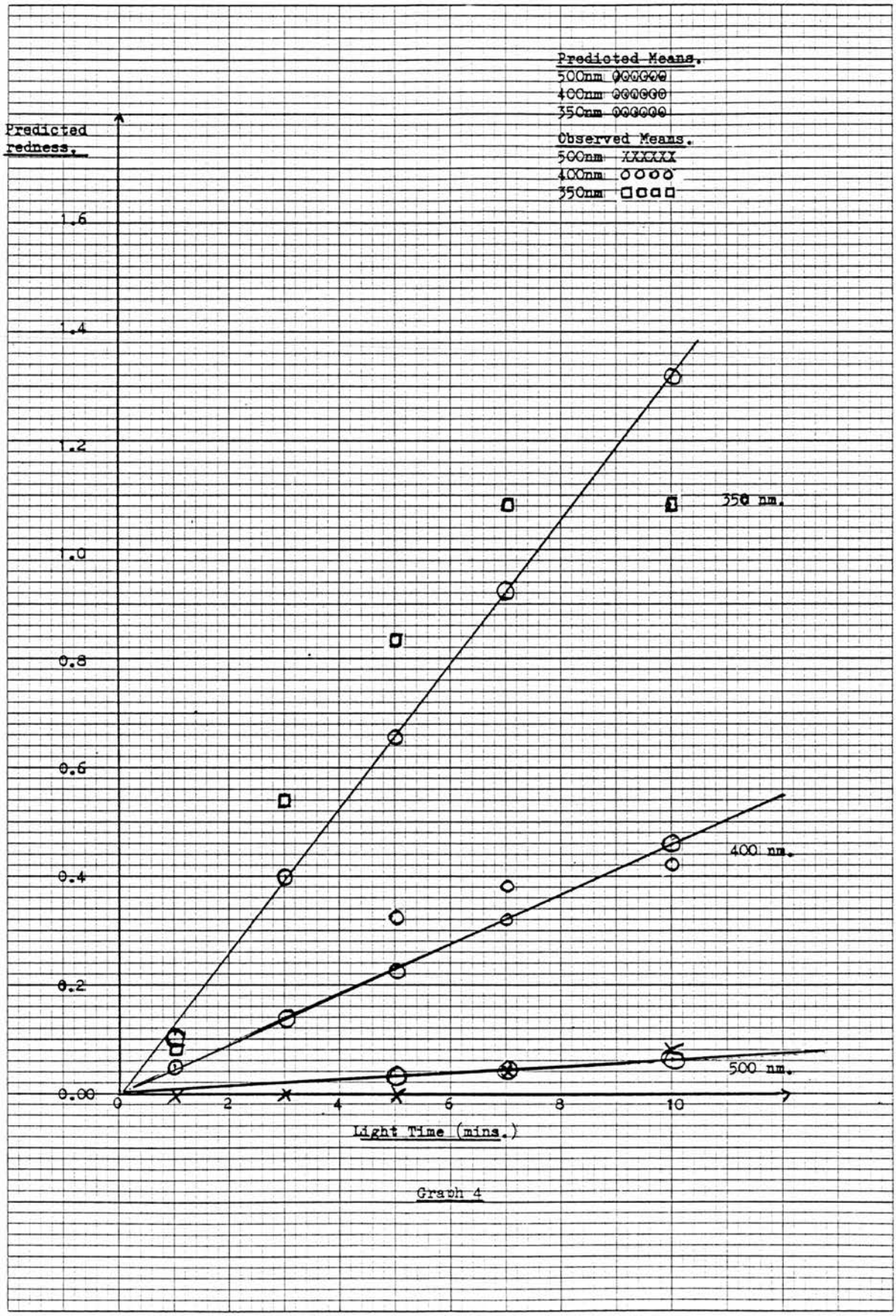
The observed and predicted mean redness gradings are shown in Table 11 and plotted in Graph 4.

Plate 10 shown a typical photopatch reaction. The mis-shapen 5minute reaction indicates movement by the subject during this exposure.

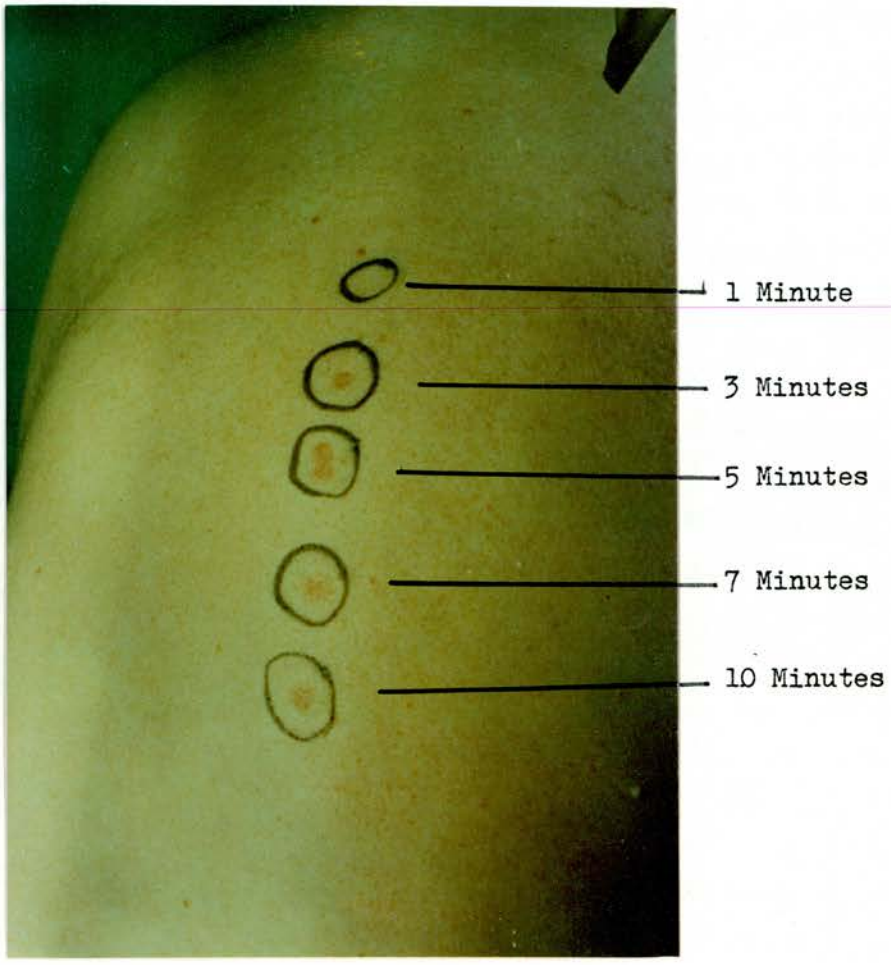
REDNESS VALUES

Light Time in Minutes	Filter Wavelength					
	500 nm		400 nm		350 nm	
	Obs.	Pred.	Obs.	Pred.	Obs.	Pred.
0	0.00	0.00	0.00	0.00	0.00	0.00
1	0.00	0.01	0.00	0.05	0.08	0.13
3	0.00	0.02	0.00	0.14	0.54	0.40
5	0.00	0.03	0.33	0.23	0.83	0.66
7	0.04	0.04	0.38	0.32	1.08	0.92
10	0.08	0.06	0.42	0.46	1.08	1.32

Table 11


Predicted Means.
500nm ◊◊◊◊◊◊
400nm ◊◊◊◊◊◊
350nm ◊◊◊◊◊◊
Observed Means.
500nm XXXXXX
400nm ○○○○
350nm □□□□
Predicted redness.
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.00
0
2
4
6
8
10
350 nm.
400 nm.
500 nm.
Light Time (mins.)

Graph 4



(Subject MC) displaying the photopatch reaction

Plate 10

(i) Action Spectroscopy

As the experiments with the 3 filters indicated that a part of the action spectrum of benzanthrone lay in the 350 - 400 nm region further studies were undertaken to delineate more precisely the action spectrum of benzanthrone photosensitivity.

A group of subjects was taken to the Photobiology Unit of the Department of Dermatology at Dundee for further photopatch investigations. There, photopatch testing was carried out using a Xenon light source coupled to a diffraction grating monochromator which facilitated selection of appropriate narrow wavebands in the long UV and visible wavebands. This enabled the threshold exposure dose producing this immediate erythema to be elicited.

Method

Over a period of several weeks 10 subjects were each patch tested for 2 hours with a concentration of 2% benzanthrone in PMF, each subject having 24 patches applied to the infra scapular zones. The subjects were then taken from the works at Grangemouth to the Photobiology Unit at the Department of Dermatology at Dundee. At the Photobiology Unit they were photopatch tested in the range 360 - 425 nm with exposure times ranging from 12 seconds to 5 minutes.

Results

The results were recorded according to the grading and classification scale and are shown in Table 12 (pages 107-110) which also depicts the wavelengths, the exposure times and the computed exposure dose expressed in $mJcm^{-2}$ used. - (See methods page 77)

Plate 11 shows the immediate post exposure reaction to light in the subject JA.

SUBJECT	WAVELENGTH nm	TIME SECS.	DOSE $mJcm^{-2}$	IMMEDIATE POST EXPOSURE REACTION TO LIGHT
MAC	365	30"	714	0
		1'0"	1428	0
		2'0"	2856	0.5
		4'0"	5712	1
	380	30"	780	0
		1'0"	1560	0
		2'0"	3120	0.5
		4'0"	6240	1
	387.5	30"	762	0
		1'0"	1524	0
		2'0"	3048	0
		4'0"	6096	1
	395	30"	756	0
		1'0"	1512	0
		2'0"	3024	1
		4'0"	6048	2
	410	30"	750	0
		1'0"	1500	0
		2'0"	3000	1
		4'0"	6000	2
	425	30"	762	0
		1'0"	1524	0
		2'0"	3048	1
		4'0"	6096	2
SAW	365	30"	714	0
		1'0"	1428	0
		2'0"	2856	0
		4'0"	5712	0.5
	380	30"	780	0
		1'0"	1560	0
		2'0"	3120	0
		4'0"	6240	2
	387.5	30"	762	0
		1'0"	1524	0
		2'0"	3048	0.5
		4'0"	6096	1
	395	30"	756	0
		1'0"	1512	0
		2'0"	3024	0.5
		4'0"	6048	1
	410	30"	750	0
		1'0"	1500	0
		2'0"	3000	1
		4'0"	6000	2
	425	30"	762	0
		1'0"	1524	0
		2'0"	3048	0
		4'0"	6096	1
JS	365	3'0"	4428	0
		6'0"	8856	0.5
	380	30"	798	0
		45"	1197	0
		1'45"	2793	0
		2'30"	3990	0
		5'0"	7980	0
	395	15"	393	0
		30"	786	0
		30"	786	0
		1'0"	1572	1
		2'0"	3144	2
		4'0"	6288	2
		5'0"	7860	2
	400	30"	768	0
		45"	1152	0
		1'0"	1536	1
		1'30"	2304	1
		2'0"	3072	2

Table 12

SUBJECT	WAVELENGTH n m	TIME SECS.	DOSE $mJcm^{-2}$	IMMEDIATE POST EXPOSURE REACTION TO LIGHT
RR	365	2'0"	2808	0
		4'0"	5616	0
	370	2'0"	2707	0
		4'0"	5414	0
	375	2'0"	2722	0
		4'0"	5444	0.5
	380	2'0"	2837	0
		4'0"	5674	0.5
	385	2'0"	2635	0
		4'0"	5270	0.5
	390	2'0"	2688	0
		3'0"	4032	0.5
		4'0"	5376	0.5
		4'0"	5376	0.5
		5'0"	6720	1
		6'0"	8064	2
	395	2'0"	2791	0.5
		4'0"	5582	1
	400	2'0"	2652	0.5
		4'0"	5304	1
	405	2'0"	2748	0
		4'0"	5496	0
	410	2'0"	2520	0
		4'0"	5040	0
BM	370	2'10"	5600	0
		2'38"	6800	0
		3'10"	8200	0
		3'52"	10000	0
	380	0'44"	2200	0
		1'18"	3900	0
		1'35"	4700	2
		1'53"	5600	2
		2'17"	6800	2
	385	0'44"	2200	0
		1'18"	3900	1
		1'53"	5600	1
		2'17"	6800	2
	390	0'46"	2200	0
		1'18"	3900	2
		1'58"	5600	2
	400	1'22"	3900	0
		1'39"	4700	2
		1'58"	5600	2
		2'23"	6800	2
		2'53"	8200	2
		3'31"	10000	2
TW	370	1'14"	5600	0
		1'30"	6800	0
		1'48"	8200	0
		2'12"	10000	1
		2'39"	12000	1
	380	0'28"	2200	0
		0'49"	3900	0
		1'0"	4700	1
		1'11"	5600	2
		1'26"	6800	3
	385	0'29"	2200	0
		0'51"	3900	0
		0'51"	3900	0
		1'14"	5600	2
	390	0'15"	1200	0
		0'22"	1800	1
		0'28"	2200	1
		0'49"	3900	2
	400	0'51"	3900	0
		1'11"	5600	0
		1'48"	8200	1
		2'12"	10000	1

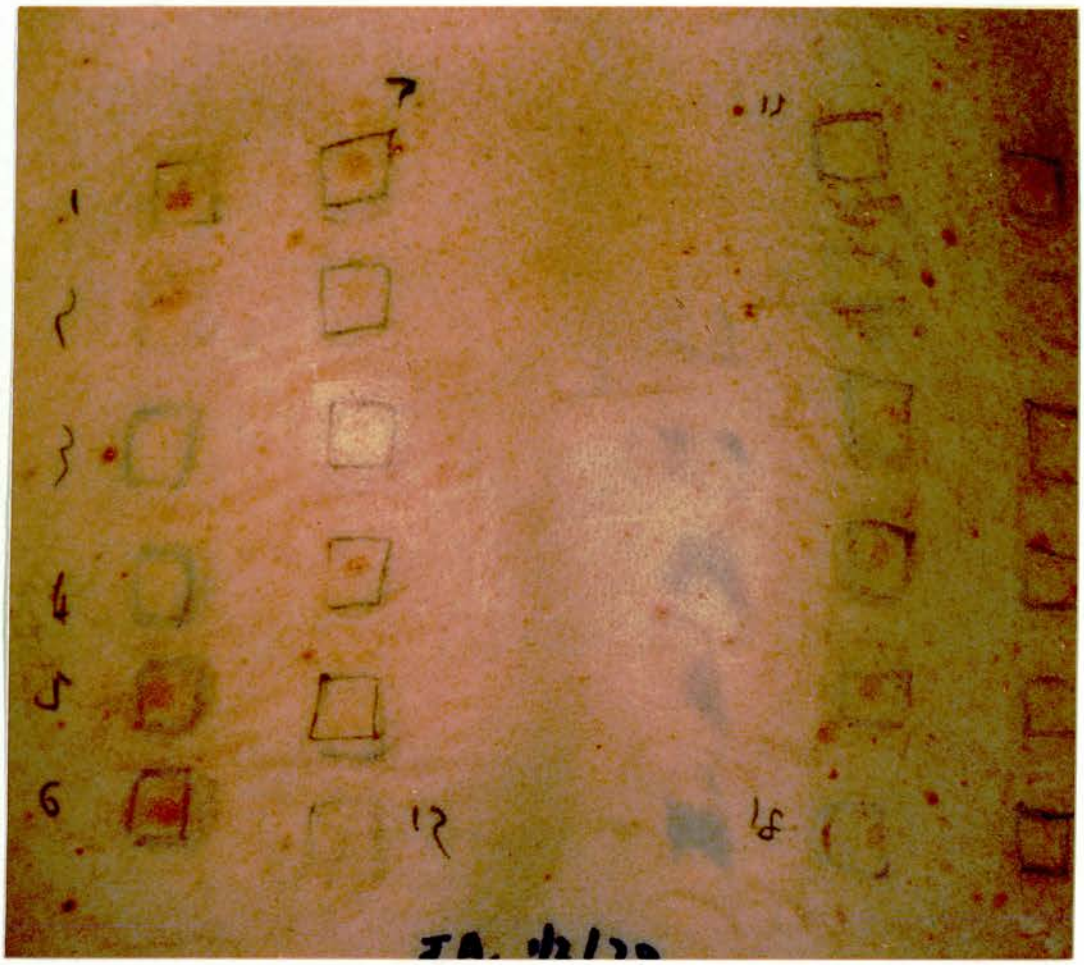
Table 12

SUBJECT	WAVELENGTH n m	TIME SECS.	DOSE $mJcm^{-2}$	IMMEDIATE POST EXPOSURE REACTION TO LIGHT
JA	370	2'15"	4700	0
		2'40"	5600	1
		3'15"	6800	2
	380	0'36"	1500	0
		0'44"	1800	2
		0'54"	2200	2
		1'36"	3900	2
		1'55"	4700	2
	385	0'36"	1500	0
		0'44"	1800	2
		0'54"	2200	2
		1'36"	3900	2
	390	0'36"	1500	0
		0'44"	1800	2
		0'54"	2200	2
		1'36"	3900	2
	400	1'6"	2700	0
		1'21"	3300	2
		1'36"	3900	2
		1'55"	4700	2
MK	360	0'53"	3900	1
		1'33"	6800	2
		2'17"	10000	2
	370	0'37"	2700	0
		1'4"	4700	3
		1'56"	8200	3
		2'17"	10000	3
	380	0'13"	1000	0
		0'24"	1800	2
		0'37"	2700	3
		0'53"	3900	3
		1'4"	4700	3
		1'33"	6800	3
	385	0'16"	1200	0
		0'37"	2700	3
		1'4"	4700	3
		1'33"	6800	3
	390	0'13"	1000	0
		0'24"	1800	2
		0'45"	3300	3
		1'17"	5600	3
	400	0'25"	1800	0
		0'38"	2700	2
		1'7"	4700	3
JA	360	0'43"	3900	1
		1'15"	6800	2
		1'50"	10000	2
	370	0'25"	2200	0
		0'45"	3900	1
		1'19"	6800	1
		1'56"	10000	2
	380	0'13"	1200	1
		0'24"	2200	1
		0'43"	3900	2
		1'15"	6800	2
		1'50"	10000	2
	385	0'12"	1200	2
		0'23"	2200	2
		0'41"	3900	2
		1'11"	6800	2
	390	0'12"	1200	2
		0'23"	2200	2
		0'41"	3900	2
		1'11"	6800	2
	400	0 12"	1200	0
		0'22"	2200	0.5
		0'39"	3900	2
		1'8"	6800	2

Table 12

SUBJECT	WAVELENGTH n. m	TIME SECS.	DOSE $mJcm^{-2}$	IMMEDIATE POST EXPOSURE REACTION TO LIGHT
SAW	360	1'20"	5600	0
		1'37"	6800	1
		2'2"	8200	2
		2'33"	10000	2
	370	1'37"	6800	0
		1'57"	8200	0
		2'23"	10000	0
		2'52"	12000	2
	380	1'10"	4700	0
		1'23"	5600	0.5
		1'41"	6800	1
		2'2"	8200	2
		2'29"	10000	2
	385	1'37"	6800	0
		1'57"	8200	0.5
		2'23"	10000	3
		2'52"	12000	3
	390	1'33"	6800	0
		1'53"	8200	0.5
		2'17"	10000	0.5
		2'45"	12000	2
	400	2'59"	12000	0
		3'44"	15000	1
		4'29"	18000	4

Table 12



(JA). Photograph of twenty four patch tests on the infra scapular area with varying wavelengths and exposure times.

Plate 11

(j) Results

Examination of the minimally effective dose figures for each individual tested shows that there is a broad spectrum of effect from around 360 nm to beyond 400 nm. Because of the limitations in numbers of subjects and back space available for testing, it was not possible to obtain minimal-effect dose data for all subjects at all wavelengths. Moreover, there is a relatively high degree of individual variation in response levels. However, an approximate action spectrum for benzanthrone phototoxicity in human skin was constructed from the figures and is shown in Table 13 and Graph 5. This was done by taking the mean of the minimal effective dose at each wavelength (where individual values were not obtained, the nearest dose was used for the calculation). The reciprocal of the mean was calculated and the result for each wavelength expressed as a percentage of the most effective wavelength value. The action spectrum is thus a plot of relative effectiveness. The general impression of a wide peak is born out by this treatment but there is a maximum at around 395 nm. The remainder of the action spectrum appears to consist of minor peaks and troughs which are probably a result of test variation.

The absorption of radiation by a chemical compound depends on its atomic and molecular structure, giving an absorption of wavelengths which is specific for each compound. Each compound causing a photocontact dermatitis therefore has a characteristic absorption spectrum.

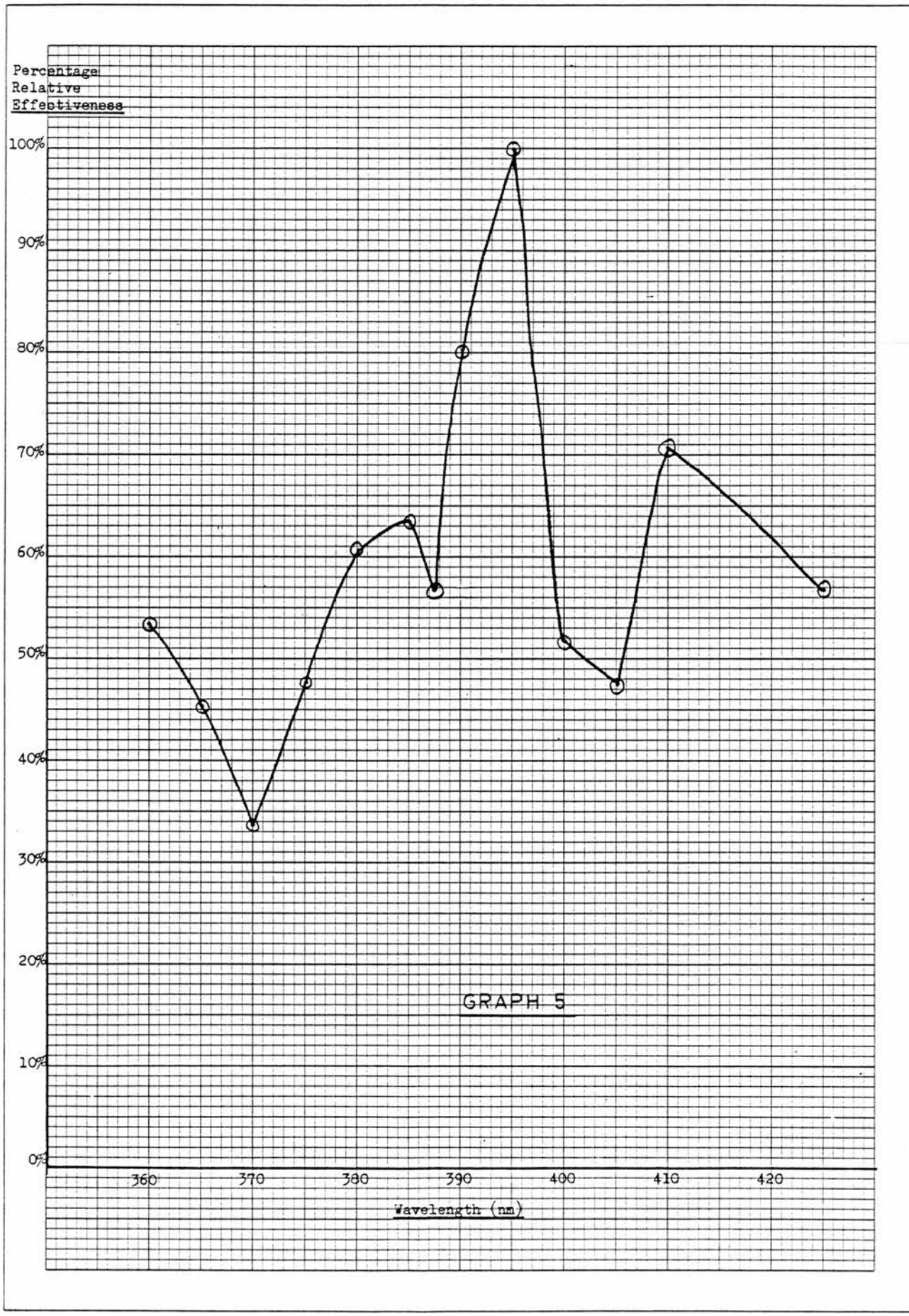
The absorption spectrum of benzanthrone was obtained with a Perkin Elmer 200 spectrophotometer and is displayed in Figure 17.

This shows an absorption peak around 390 nm. The results of the monochromator testing show that the action spectrum for benzanthrone phototoxicity also peaks around 390 nm, matching the absorption spectrum well in this respect.

MINIMAL EFFECTIVE DOSE RESULTS AND CALCULATIONS FOR ACTION SPECTRUM

360	365	370	375	380	385	387.5	390	395	400	405	410	425	Wavelength
3900	2856	5414	5444	3120	5270	6096	4032	3024	1536	5496	3000	3048	
3900	5712	9976		6240	3900	3048	3900	3024	2652		3000	6096	
6800	8856	10000		7980	5600		1800	1572	4700		5040		Minimal effective
	5616	10000		5674	1800		1800	2791	8200				dose in mJ/cm^2
		5600		4700	2700		1800		3300				
		4700		4700	1200		1200		2700				
		3900		1800	8200		8200		2200				
		12000		1800					15000				
				1200									
				5600									
4867	5760	7699	5444	4281	4097	4572	3247	2603	5036	5496	3680	4572	Mean
2.05	1.74	1.299	1.84	2.34	2.44	2.19	3.08	3.84	1.99	1.82	2.72	2.19	Reciprocal x 10^{-4}
53.4	45.2	33.8	47.8	60.8	63.5	56.9	80.2	100	51.7	47.4	70.7	56.9	%

Table 13


Percentage Relative Effectiveness
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
360
370
380
390
400
410
420
Wavelength (nm)

GRAPH 5

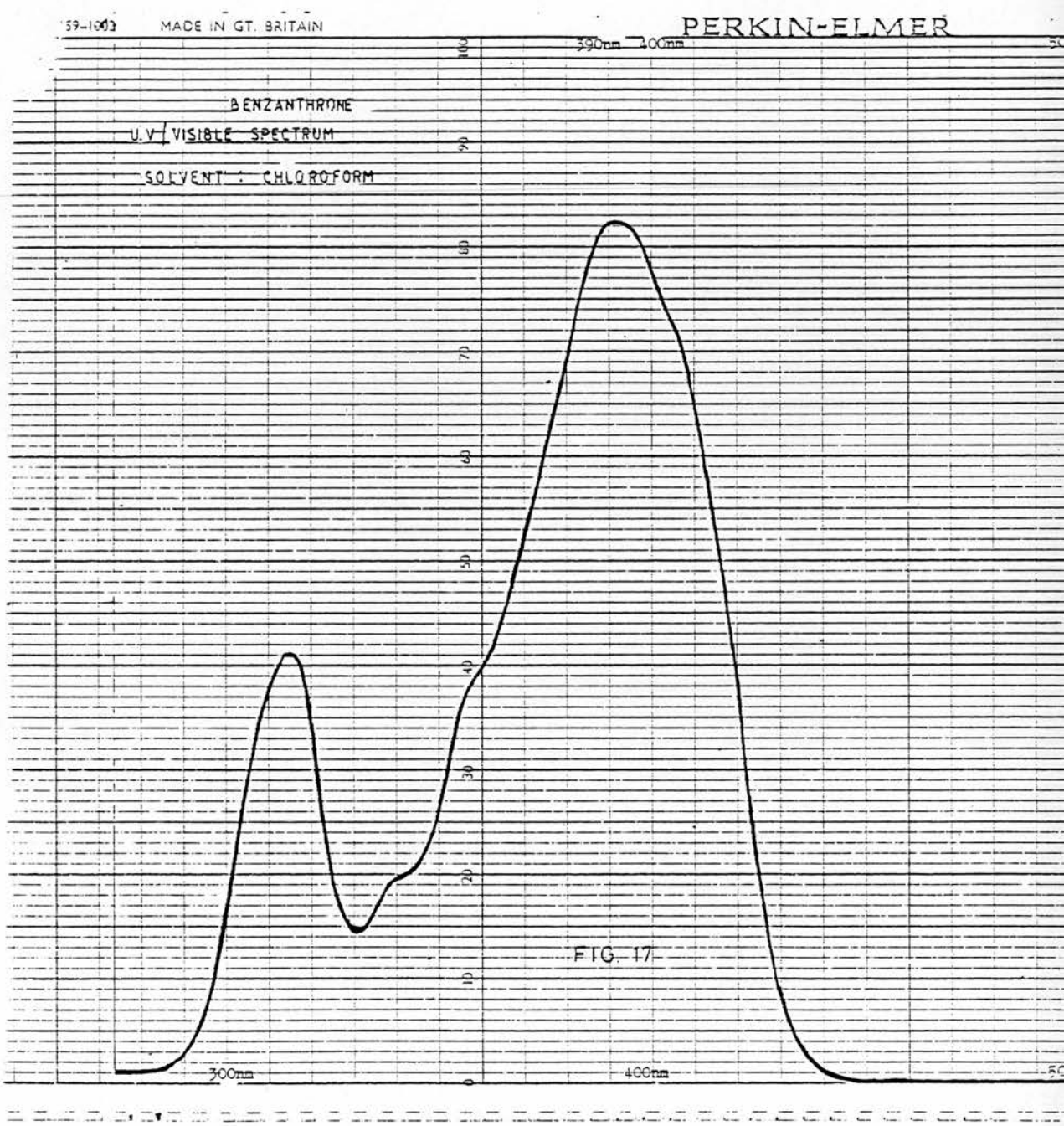

MADE IN GT. BRITAIN
PERKIN-ELMER
390nm
400nm
BENZANTHRONE
U.V. VISIBLE SPECTRUM
SOLVENT : CHLOROFORM
100
90
80
70
60
50
40
30
20
10
0
300nm
400nm

FIG. 17

Chapter V

STUDY OF ASCORBIC ACID IN INHIBITING THE PHOTOTOXIC EFFECT OF BENZANTHRONE

Including

1. INTRODUCTION

2. INVESTIGATION AND RESULTS
   - (a) Subjects
   - (b) Renal Function
   - (c) Results
   - (d) Plasma and Leucocyte Ascorbic Acid Levels
   - (e) Results
   - (f) Patch and Photopatch Testing
   - (g) Results
   - (h) Statistical Analytical Techniques
     - (i) Significance Tests
     - (ii) Paired Comparison Tests
     - (iii) "Sign Test"

3. RESULTS AND CONCLUSIONS.

ASCORBIC ACID

1. INTRODUCTION

The ability of mammals such as the rat, rabbit, mouse, dog and goat to synthesize ascorbic acid contrasts with the need for an exogenous supply of this vitamin by other mammals such as man and guinea pig. It is presumed that man is unable to synthesize ascorbic acid because he is unable to convert d-glucurono-γ-lactone and L-glucurono-γ-lactone to this vitamin.

Studies with C^{14} labelled L-ascorbic acid have shown that the vitamin is oxidized to respiratory carbon dioxide in rats and guinea pigs, but not in humans. In fact ascorbic acid is metabolised slowly in humans and has a half-life of about sixteen days, compared to about four days in the guinea pig. Hence humans, whose diet contains no vitamin C take three to four months to develop scurvy whereas guinea pigs become scorbutic in only three weeks.

The plasma ascorbic acid provides a reasonable index of dietary intake though it is a poor guide to the tissue stores. Although it is possible for patients with scurvy to have an undetectable plasma ascorbic acid level the converse is not true. Leucocyte ascorbic acid reaches a minimum at about the same time as clinical evidence of scurvy appears and thus seems to give a good indication of the body's stores of this vitamin.

There is general agreement that a daily intake of 500 mg of ascorbic acid for a prolonged period does not usually produce toxic effects. However, the administration of mega doses of ascorbic acid, ranging from 1 gram to 10 grams daily, can give rise to undesirable side effects. Lamden & Chrystowski (1954) showed that ingested ascorbic acid is metabolised in humans to oxalate and

excreted mainly in the urine. Although it has been inferred that oxaluria might produce calcium oxalate calculi; only one case of a healthy individual developing a stone after taking a short course of ascorbic acid treatment has been reported (Briggs et al 1973). Takiguchi et al (1966) found no significant increase in urinary oxalate excretion on administration of up to 2 grams of ascorbic acid for up to six months.

Singh & Zaidi (1969) concluded that oral administration of ascorbic acid (50 mg/kg) appeared to inhibit the dermal changes in mice caused by local application of benzanthrone. Subsequently Pandaya, Singh & Joshi (1970) showed that the toxic action of benzanthrone on scorbutic animals could be reversed by ascorbic acid and accordingly, with these experiments in mind, an attempt was made to study the action of ascorbic acid in inhibiting the phototoxic effect of benzanthrone in humans.

2. INVESTIGATION AND RESULTS

(a) Subjects

A study was made of 26 male volunteers ranging in age from 24 to 56 and whose work-related exposure to benzanthrone ranged from 2 to 30 years. All the subjects had experienced the symptoms of phototoxicity caused by benzanthrone and several had, on occasions, been unable to follow their customary outdoor pursuits.

(b) Renal Function Analysis

Because of the possibility of inducing calcium oxalate stone formation in the subjects, due to mega doses of ascorbic acid, an assessment was made of their renal function. A 24 hour urine sample was collected from each of the 26 subjects as was a 10 ml venous blood sample. The renal function analysis was carried out by the Department of Clinical Chemistry at Falkirk Royal Infirmary.

(c) Results

The results of the renal function analysis are displayed in Tables 14 and 15.

RENAL FUNCTION ANALYSIS

SUBJECT	AGE	VOLUME ml	UREA m.mol/l	URCRT m.mol/24hr	PLSCRT µmol/l	CLEAR ml/min
PB	32	1700	5.8	11.1	100	131
RS	43	990	4.2	17.0	90	130
GR	34	2210	4.3	10.0	90	171
CC	56	1370	5.4	9.0	90	95
RR	36	2060	6.7	4.4	80	79
RH	37	820	3.8	18.2	70	148
JA	44	1220	4.5	10.1	90	96
MK	37	900	5.5	19.0	110	108
WA	46	1640	5.9	12.2	100	139
AH	41	2330	5.1	6.6	90	119
AC	51	1840	5.0	8.1	80	129
RR	53	1590	4.5	12.5	90	153
HM	49	1250	4.8	13.7	80	149
MM	49	1380	3.5	8.8	80	105
CF	45	1540	5.0	7.4	60	132
CV	24	1320	3.7	7.4	80	85
IF	39	1860	4.1	13.2	100	170
JS	32	1580	5.6	10.6	80	145
RM	40	1540	3.4	11.2	70	171
MH	40	1300	4.2	12.0	80	135
GB	38	1570	4.9	11.6	100	126
JB	50	2060	5.1	6.7	110	87
RD	33	960	4.3	13.6	70	130
DT	52	1660	5.9	10.0	100	115
AH	40	920	5.4	15.8	90	112
TR	39	2130	3.0	7.8	90	128

Normal Values Falkirk Infirmary Biochemical Laboratory

URCRT = Urine Creatinine — Blood Urea 2.5 - 8.0 m/mol/l
PLSCRT = Plasma Creatinine — Plasma Creatinine 60 - 130 µmol/l.
CLEAR = Creatinine clearance — Urine Creatinine 9 - 18 m/mol 24hrs
UREA = Blood Urea — Creatinine Clearance 80-110 ml/min.

Table 14

SUMMARY OF
RENAL FUNCTION ANALYSES

VARIABLE	MEAN	S.D.	MINIMUM	MAXIMUM
AGE	41.54	7.71	24	56
VOLUME	1528.5	424.5	820	2330
UREA	4.75	0.90	3.0	6.7
URCRT	11.08	3.67	4.4	19.0
PLSCRT	87.31	12.51	60.0	111.0
CLEAR	126.5	26.0	79.0	171.0

URCRT = URINE CREATININE

PLSCRT = PLASMA CREATININE

CLEAR = CREATININE CLEARANCE

Table 15

(d) Plasma and Leucocyte Ascorbic Acid Levels

A further blood sample was taken from each of the 26 subjects and analysed for the "pre-course" levels of ascorbic acid present in the leucocytes and plasma. This analysis was carried out by the Department of Clinical Chemistry at Ninewells Hospital, Dundee using the method of Denson & Bowers (1961).

The subjects were then given 500 mg ascorbic acid Q.I.D. (Redoxon 0.5g) orally over a two week period in order to saturate their body stores. At the end of this period a further blood sample was taken for comparison analysis with the "pre-course" levels of ascorbic acid in the plasma and leucocytes.

(e) Results

Several subjects developed sore tongues and diarrhoea during the two week period.

The results of the "pre-dose" and "post-dose" levels of plasma and leucocyte ascorbic acid are shown in Table 16.

		DAY 0		DAY 14	
SUBJECT	AGE	PRE DOSE VIT C PLASMA	LEUCOCYTE	POST DOSE VIT C PLASMA	LEUCOCYTE
PB	32	14.5 µg/ml	33.8 µg/WBC	8.7µg/ml	32.9 µg/WBC
BS	43	14.5 "	23.2 "	14.5 "	37.4 "
GR	34	8.5 "	28.8 "	10.9 "	30.4 "
CC	57	11.5 "	30.7 "	19.8 "	40.7 "
RR	36	4.2 "	21.3 "	14.3 "	27.8 "
RH	37	1.5 "	20.9 "	22.1 "	28.1 "
JA	44	2.5 "	14.2 "	5.3 "	18.5 "
MK	37	5.0 "	24.3 "	11.9 "	20.4 "
WA	46	3.7 "	18.1 "	20.8 "	23.1 "
AH	41	6.5 "	21.6 "	15.7 "	20.1 "
AC	51	5.7 "	25.5 "	17.5 "	29.0 "
RR	53	1.7 "	9.9 "	3.5 "	15.7 "
HM	49	1.3 "	15.3 "	20.8 "	37.3 "
MM	49	13.5 "	26.5 "	21.0 "	32.5 "
CF	45	3.5 "	23.4 "	17.3 "	24.6 "
CV	24	12.7 "	23.7 "	20.8 "	28.3 "
DF	39	3.5 "	23.0 "	10.4 "	35.6 "
JS	32	5.9 "	26.2 "	16.8 "	27.1 "
RM	40	6.8 "	28.0 "	14.1 "	23.5 "
NH	40	9.2 "	30.6 "	18.4 "	33.8 "
GB	38	5.5 "	27.0 "	13.6 "	28.5 "
JB	50	10.2 "	26.8 "	14.0 "	22.4 "
RD	33	14.5 "	30.8 "	20.2 "	27.2 "
DT	52	3.6 "	26.1 "	15.6 "	32.4 "
AH	40	3.7 "	26.7 "	19.3 "	26.3 "
TR	39	7.2 "	20.5 "	18.5 "	17.2 "

Normal Values : Department of clinical chemistry.
Ninewells Hospital, Dundee

Leucocytes	21 - 53 µg/10^8 WBC
Plasma	4 - 15 µg/ml

Table 16

The mean pre-dose and post-dose ascorbic acid levels in the plasma and leucocytes are given in Table 17 and the mean increases between the pre-dose levels and post-dose levels are given in Table 18.

Effect of Vitamin C on Plasma and Leucocytes

VARIABLE	NUMBER OF SUBJECTS	MEAN	S.D.	MINIMUM	MAXIMUM
Pre-dose plasma µg/ml	26	6.96	4.32	1.3	14.5
Post-dose plasma µg/ml	26	15.61	4.91	3.5	22.1
Pre-dose leucocyte µg/WBC	26	24.11	5.49	9.9	33.8
Post-dose leucocyte µg/WBC	26	27.72	6.53	15.7	40.7

Table 17

VARIABLE	NUMBER OF SUBJECTS	MEAN	S.D.	MINIMUM	MAXIMUM
Increase in plasma µg/ml	26	8.65	5.96	-5.8	20.6
Increase in leucocyte µg/WBC	26	3.61	6.28	-4.5	22.0

Table 18

The data was analysed using a statistical paired-comparison test, which showed that the increase in leucocyte levels of ascorbic acid is highly significant ($p < 0.01$) while the increase in plasma levels of ascorbic acid is very highly significant ($p < 0.0001$); these increases being attributable to the vitamin C dosing.

(f) Patch and Photopatch Testing

On day fourteen, at the cessation of the ascorbic acid dosage, the 26 subjects were patch tested with 2% benzanthrone in PMF for a period of 1 hour. Each patch was then irradiated with light from a 500W Xenon lamp using a Schott cut-off filter GG 375 for 3,5,7 and 10 minutes and the results read, graded and classified immediately and 2 hours post exposure.

A period of 4 weeks was now allowed to elapse; during this "wash out" period, the highly artificially-induced values of plasma and leucocyte ascorbic acid returned to their pre-dose levels (McLaren 1980 personal communication).

On day forty two the same group of 26 subjects were again patch tested with 2% benzanthrone in PMF for a period of 1 hour. Each patch being again irradiated with light from a 500W Xenon arc lamp using a Schott cut-off filter GG 375 nm for 3, 5, 7 and 10 minutes and the results read, graded and classified immediately and 2 hour post exposure. Figure 18 shows the dose and photo-patch schedule from day 0 to day 42.

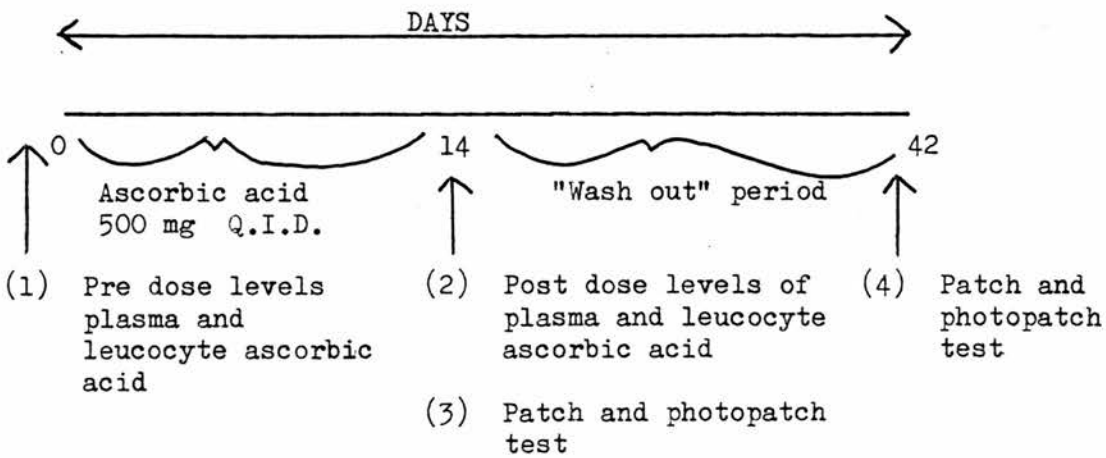


Fig. 18

(g) Results

Tables 19 and 20 show the photopatch results obtained with normal and increased levels of ascorbic acid.

PHOTOPATCH RESULTS OBTAINED WITH INCREASED LEVELS OF ASCORBIC ACID

DATE	SUBJECT	PHOTOPATCH EXPOSURE GRADING REACTION AT 375 NM							
		IMMEDIATE				2 HOURS			
		3 mins	5 mins	7 mins	10 mins	3 mins	5 mins	7 mins	10 mins
4.12.80.	HM (1)	0	0	0	0	0	0	0	0
"	MK (2)	1	2	2	2	0	2	2	2
"	JS (3)	0	0	0	0	0	0	0	0
"	PB (4)	0	0	0	0	0	0	0	0
"	CV (5)	0	1	2	2	0	0	2	2
"	RH (6)	0	0	0	0	0	0	0	0
"	AH (7)	1	1	1	1	0	0	0	1
5.12.80.	RS (8)	0	0	0	0	0	0	0	0
"	JA (9)	0	0	2	2	0	0	2	1
"	IF (10)	0	0	0	0	0	0	0	0
"	CC (11)	0	0	0	0	0	0	0	0
"	RR (12)	0	0	0	0	0	0	0	0
"	MM (13)	0	0	0	0	0	0	0	0
"	GR (14)	0	0	0	0	0	0	0	0
"	JB (15)	0	0	0	0	0	0	0	0
10.12.80.	RD (16)	0	0	0	0	0	0	0	0
"	TR (17)	0	0	0	0	0	1	1	1
"	GB (18)	0	0	0	0	0	0	0	0
"	CF (19)	0	0	0	0	0	0	0	0
"	AH (20)	2	2	2	2	1	1	1	1
"	WA (21)	2	2	2	2	1	1	1	1
"	AC (22)	0	0	0	0	0	0	0	0
11.12.80.	RM (23)	1	1	1	1	2	2	1	1
"	DT (24)	0	0	0	0	0	0	0	0
12.12.80.	NH (25)	1	1	1	1	0	0	1	1
15.12.80.	RR (26)	0	0	0	0	0	0	0	0

TABLE 19

PHOTOPATCH RESULTS OBTAINED WITH NORMAL LEVELS OF ASCORBIC ACID

DATE	SUBJECT	PHOTOPATCH EXPOSURE GRADING REACTION							
		IMMEDIATE				2 HOURS			
		3 mins	5 mins	7 mins	10 mins	3 mins	5 mins	7 mins	10 mins
13.1.81.	HM (1)	0.5	1	1	1	0	0.5	0.5	0.5
15.1.81.	MK (2)	2	2	3	3	2	2	2	2
14.1.81.	JS (3)	0.5	1	1	1	0	1	1	1
9.1.81.	PB (4)	0	0	0.5	1	0	0	0	0
8.1.81.	CV (5)	0	1	2	3	0	0	1	2
19.1.81.	RH (6)	1	2	2	2	0	0	1	1
9.1.81.	AH (7)	0	1	1	1	0	0	0	0
"	RS (8)	0	0.5	1	1	0	0	0	0
15.1.81.	JA (9)	2	2	2	2	1	2	2	2
12.1.81.	IF (10)	0	0.5	2	2	0	0	0.5	1
9.1.81.	CC (11)	0.5	1	1	1	0	0	0	0
12.1.81.	RR (12)	0	0	0	0	0	0	0	0
14.1.81.	MM (13)	0	1	1	1	0	1	1	1
9.1.81.	GR (14)	2	2	2	2	0	0	1	2
12.1.81.	JB (5)	0	1	2	2	0	0.5	1	1
9.1.81.	RD (16)	0	1	1	1	0	0	0.5	1
"	TR (17)	0.5	1	1	1	0	1	1	1
12.1.81.	GB (18)	2	2	2	2	0	0.5	1	1
14.1.81.	CF (19)	1	1	1	1	1	1	1	1
"	AH (20)	1	1	1	1	0.5	0.5	0.5	1
"	WA (21)	1	1	2	2	0.5	1	1	2
"	AC (22)	1	1	1	1	0	0.5	0.5	0.5
9.1.81.	RM (23)	2	3	3	3	1	2	2	3
19.1.81.	DT (24)	0.5	0.5	1	1	0	0	0	0
12.1.81.	NH (25)	2	2	2	2	1	1	2	2
15.1.81.	RR (26)	0.5	1	2	2	0	0.5	1	1

TABLE 20.

(h) Statistical Analytical Techniques

(i) Significance Tests

Statistical significance testing (or hypothesis testing) is a powerful tool for assessing whether experimentally observed variations from the norm are only the result of random fluctuations or are an indication of a real change in the underlying situation. These tests are based on assessing the probability of such variations being observed if there has not been a change in the underlying situation. The results of a significance test are usually reported by reference to this probability. If the probability is sufficiently small then it is almost certain that a real change has occurred. In most situations a probability of less than 5% (i.e. 0.05) is considered sufficiently small to indicate that a real change has occurred: the smaller the probability, the greater the likelihood of the change.

(ii) Paired Comparison Test

This test is used to assess whether a particular "treatment", which has been applied to all the subjects under investigation, has induced a real change in some measurable response. The test uses the observed difference between the pre-treatment response and the post-treatment response for each subject and assesses the probability that such differences would have been observed if the application of the treatment had no effect on the response. If this probability is small (i.e. if the test gives a "significant" result) the implication is that a real change in the response has been induced by the application of the treatment.

(iii) "Sign" Test

The "sign" test is also used to assess whether a "treatment" has

induced a real change in a response. It can be applied to situations in which the response is not measurable but can be given a grading or ranking. The observed change can then be categorised as simply "increase", "decrease" or "no change" usually coded as "+", "-", or "o". The test assesses whether the proportion of "+"'s and "-"'s is probable if no real change has occurred. If there is a significantly disproportionate number of "+"'s (or "-"'s) the implication is that a real change in the response has been induced by the application of the treatment.

3. RESULTS AND CONCLUSIONS

Effects of Ascorbic Acid on Photopatch Testing

Table 21 collates the redness grades of the 26 subjects immediately after exposure, for both the normal and increased levels of Vitamin C, in the form of frequency distributions. The clearly observable pattern in these results is one of reduced redness grades for the increased Vitamin C levels. To confirm this observation two statistical tests were applied to the data -

(i) The changes in grades were assessed by a paired comparison test : this test requires the assumption that the redness grades are parametric measurements, which has been necessary for the previous regression analyses, but may not be entirely true.

(ii) The changes in grades were assessed by "sign" tests, a non-parametric test which is more appropriate to the data.

The results of these tests are summarised in Table 22. A "significant" result indicates that there is a significant reduction in the redness grades when subjects have increased levels of Vitamin C.

Table 23 collates the redness grades of the subjects 2 hours after exposure, again in the form of frequency distributions. This data was also analysed using paired comparison tests and "sign" tests, the results of which are summarised in Table 24.

Examination of Tables 22 and 24 reveals that the increased level of Vitamin C resulted, with one exception, in a significant reduction in the redness grades for all the exposure times used.

IMMEDIATE REACTION : COMPARISON BETWEEN PRE & POST PHOTOPATCH TESTS

PRE DOSE VITAMIN C RESULTS			POST DOSE VITAMIN C RESULTS	
GRADE OF RESPONSE	SUBJECT FREQUENCY	EXPOSURE TIME	GRADE OF RESPONSE	SUBJECT FREQUENCY
0	9	3 minutes	0	20
0.5	6	"	0.5	0
1	5	"	1	4
2	6	"	2	2
3	0	"	3	0
0	2	5 minutes	0	19
0.5	3	"	0.5	0
1	14	"	1	4
2	6	"	2	3
3	1	"	3	0
0	1	7 minutes	0	18
0.5	1	"	0.5	0
1	12	"	1	3
2	10	"	2	5
3	2	"	3	0
0	1	10 minutes	0	18
0.5	0	"	0.5	0
1	13	"	1	3
2	9	"	2	5
3	3	"	3	0

Table 21

SUMMARY

EXAMINATION TIME	EXPOSURE TIME	ASSESSMENT OF CHANGE IN REACTION AFTER DOSE	
		By t-test	By Sign test
Immediate	3 minutes	Significant at 1% level	Significant at 0.5% level
"	5 minutes	Significant at 0.1% level	Significant at 0.1% level
"	7 minutes	Significant at 0.1% level	Significant at 0.1% level
"	10 minutes	Significant at 0.1% level	Significant at 0.1% level

Table 22

DELAYED REACTION : COMPARISON BETWEEN PRE & POST PHOTOPATCH RESULTS

PRE DOSE VITAMIN C RESULTS			POST DOSE VITAMIN C RESULTS	
GRADE OF RESPONSE	SUBJECT FREQUENCY	EXPOSURE TIME	GRADE OF RESPONSE	SUBJECT FREQUENCY
0	19	3 minutes	0	23
0.5	2	"	0.5	0
1	4	"	1	2
2	1	"	2	1
3	0	"	3	0
0	11	5 minutes	0	21
0.5	6	"	0.5	0
1	6	"	1	3
2	3	"	2	2
3	0	"	3	0
0	6	7 minutes	0	18
0.5	5	"	0.5	0
1	11	"	1	5
2	4	"	2	3
3	0	"	3	0
0	6	10 minutes	0	17
0.5	2	"	0.5	0
1	11	"	1	7
2	6	"	2	2
3	1	"	3	0

Table 23

SUMMARY

EXAMINATION TIME	EXPOSURE TIME	ASSESSMENT OF CHANGE IN REACTION AFTER DOSE	
		By t-test	By Sign test
After 2 hours	3 minutes	Not Significant	Not Significant
"	5 minutes	Significant at 1% level	Significant at 1% level
"	7 minutes	" " 0.2% level	" " 0.5% level
"	10 minutes	" " 0.1% level	" " 0.1% level

Table 24

Chapter Vi

IN VITRO STUDIES OF MECHANISMS OF PHOTOSENSITIZATION

Including

1. INTRODUCTION
   - (a) General
   - (b) Light Source
   - (c) Concentration of Benzanthrone

2. CANDIDA ALBICANS
   - (a) Methods
   - (b) Results

3. PHOTOHAEMOLYSIS
   - (a) Introduction
   - (b) Methods
   - (c) Results

4. RESULTS AND CONCLUSIONS

BENZANTHRONE

1. INTRODUCTION

(a) General

Having determined that benzanthrone was photoactive *in vivo*, further experiments were carried out to demonstrate its phototoxicity *in vitro* and to investigate the mechanism.

Earlier research has produced several methods for testing phototoxicity. *In vitro*, these have included photosensitized killing of a yeast, Candida albicans (Daniels, 1965), photohaemolysis of human erythrocytes (Kahn & Fleischaker 1971 and Kochevar & Lamola 1979), and photosensitized killing of macrophages and other nucleated mammalian cells (Allison, Magnus & Young, 1966; Freeman, Murtishaw & Knox, 1970; and Wennersten, 1979).

I decided to examine the phototoxic potential of benzanthrone by the two simplest methods available (1) the phototoxic effect on Candida albicans and (2) photohaemolysis.

(b) Light Source

The same irradiation source was used for both the Candida albicans and photohaemolysis studies. Atlas 20-W 2ft. blue fluorescent tubes were chosen as their emission spectrum coincided as close as possible with the absorption spectrum of benzanthrone.

No accurate figure is available for the output of a single tube (thermopile reading at 14.5 cm 0.4mJcm^{-2}sec^{-1}), but actinometry of three such tubes in a bank, spaced 8 cm distance from one another and 14.5 cm from the surface of a culture dish set beneath the centre of the bank, gave a figure of 0.68 mJcm^{-2}sec^{-1} for radiation intensity. The actinometric method used was that of Hatchard & Parker (1956) using potassium ferrioxalate. Figure 19 shows the emission spectrum of the atlas 20-W 2ft. blue fluorescent tube.

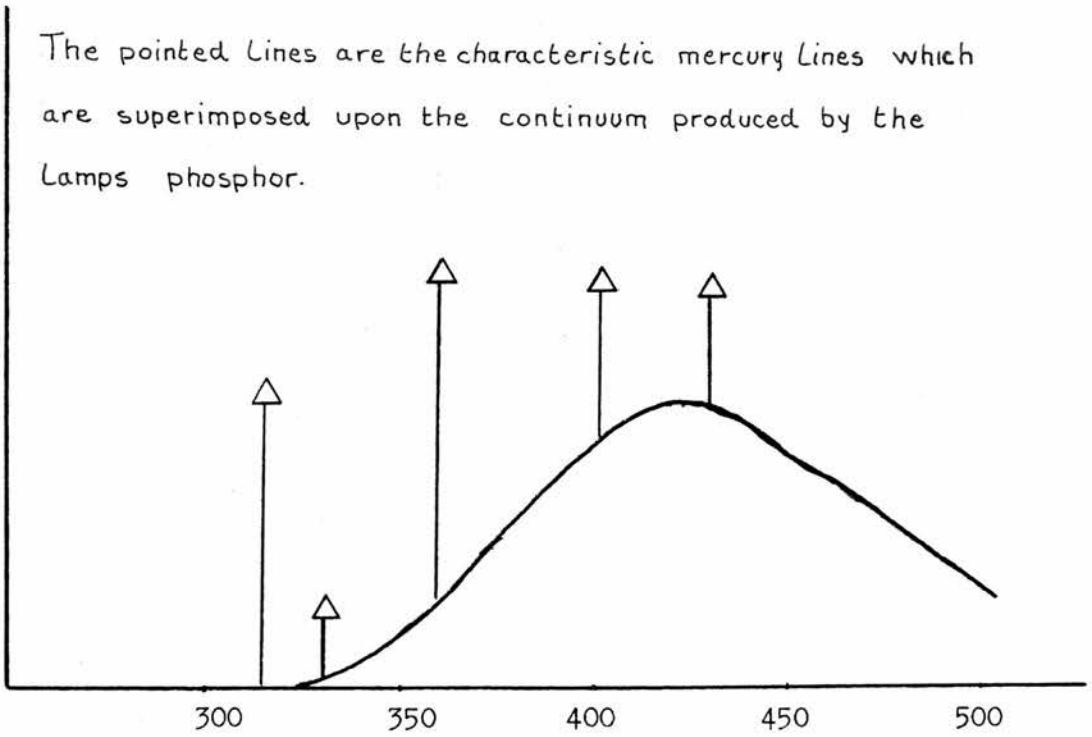


Fig. 19

(c) Concentration of Benzanthrone

The chemical was prepared as solutions in ethanol at 0.01, 0.1 and 1.0 mg/ml with controls of ethanol. (Molar concentrations 4.343×10^{-5} - 4.343×10^{-3}M).

2. EXPERIMENTAL METHODS : CANDIDA ALBICANS

Candida albicans grows readily at room temperature and inhibition of growth can be detected within 48 hours. Cultures are not affected by blue, ultra violet or black light tubes but growth is inhibited when they are irradiated by a single sun lamp tube at 14.5 cm.

(a) Method

A suspension of yeast in nutrient broth (oxoid CM2) was innoculated onto the entire surface of oxoid sabouraud and dextrose agar CN41 in petri dishes. This moistened but did not soak the plates. Paper discs 5.5 mm in diameter were impregnated with benzanthrone in a solution of ethanol to give concentrations of 0.01, 0.1 and 1.0 mg/ml with control of ethanol (molar concentrations = 4.343×10^{-5} - 4.343×10^{-3}M). The discs were placed on the agar as soon as possible after innoculation but the discs were not exposed to the appropriate radiation until yeast growth was firmly established (usually 10 hours after innoculation). Phototoxicity was shown by inhibition of yeast growth in a ring around the impregnated paper discs. The index of inhibition was taken as the distance from the edge of the impregnated paper discs to the furthest extent of inhibition.

(b) Results

Plate 12 shows the inhibition of growth of Candida by benzanthrone-impregnated filter discs. An attempt was made to culture cells from the inhibited zones in Plate 12. This failed, indicating that the cells in the inhibited area had been killed (Plate 13).

Table 25 displays benzanthrone phototoxicity to Candida obtained after 65 hours irradiation with a single blue tube and a bank of three blue tubes.

17.11.78. Inhibition of growth of Candida by benzanthrone-impregnated filter-paper discs. Marked disc was impregnated with ethanol, as a control, then dried; others (proceeding clockwise) impregnated with 0.01, 0.1, 1.0 mg/ml benzanthrone in ethanol.

Plate (12)

Plate 12

Test of plate (1) for status of Candida in inhibited zones. A sterile Sabouraud agar plate was inoculated by squares of "Parafilm" which had been pressed against the zones in plate (1), and then incubated for 2 days in dark. Results show that Candida in plate (1) was killed by the phototoxic effect. Arranged as in plate (1).

PLATE 13

Table 25

Benzanthrone Phototoxicity to Candida Albicans

1 (a) Single blue tube. Results after 65 hours irradiation; 3 replicates per treatment (light), 2 (dark).

Benzanthrone Concentration (mg/ml^{-1})	Dark (ring width, mm)	mean	Irradiated (ring width, mm)	mean
0	0,0	0	0,0,0	0
0.01	0,0	0	7,10,9	8.7
0.1	0,0	0	11,13,12	12
1.0	0,0	0	16,14,10	13.3

1 (b) 3 blue tubes. In this experiment, rings of partial inhibition were measured, since they were more sharply defined than rings of total inhibition. The irradiation time was 65 hours.

Benzanthrone Concentration (mg/ml^{-1})	Dark	Mean	Irradiated	Mean
0	0,0	0	0,0,0,0	0
0.01	0,0	0	1.5,2.0,1.5,1.5	1.6
0.1	0,0	0	4.5,5.0,3.5,3.5	4.1
1.0	0,0	0	4.5,3.0,3.5	3.7

The results therefore demonstrate a concentration dependent phototoxic effect.

3. PHOTOHAEMOLYSIS

(a) Introduction

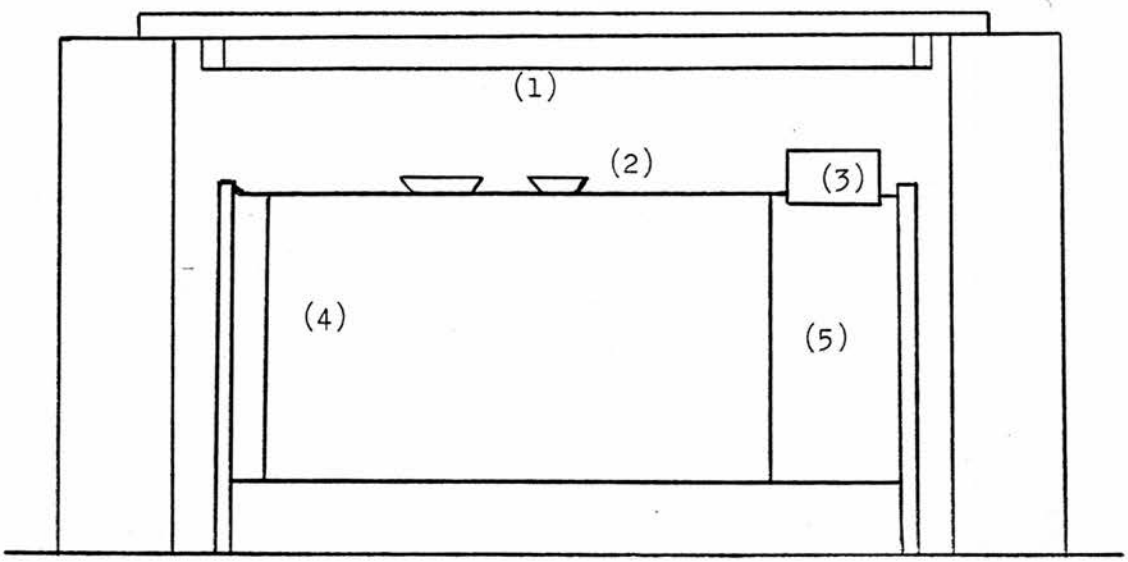
Photohaemolysis is an effect of many photosensitizing compounds and is now an established *in vitro* procedure to determine the phototoxic potential of chemicals. Many photosensitizers when irradiated in the presence of red blood cells mediate destruction of the erythrocyte membrane and release haemoglobin. This can be established and the percentage haemolysis due to the photochemical reaction calculated.

(b) Method

5 ml of freshly drawn human venous blood was placed in a heparinized blood tube which, after thorough mixing, was centrifuged at 3000 rpm for 10 minutes. The top serum level was marked, serum drawn off and discarded. The erythrocytes were covered with Michaelis buffer pH 7.4, at room temperature, to the same mark, mixed thoroughly and centrifuged. The buffer was then removed and three such washes were performed. After the last wash, the supernatant was removed and replaced with sufficient fresh buffer to restore the blood to its original volume. Blood dilutions were then prepared. One per cent each of the standard solutions was added to the buffer diluted blood (ethanol as control). The final concentration of benzanthrone was thus 0.1; 1.0 and 10.0 ug/ml (4.343×10^{-7} - 4.343×10^{-5}M). A small quantity of blood diluted in distilled water was also prepared for 100% haemolysis calibration.

Using 5 cm diameter sterilin culture dishes, 5 ml samples of well mixed diluted blood were placed in each dish and left in darkness for 30 minutes to allow the erythrocytes to settle in a monolayer thereby preventing scatter of UV and mutual shielding.

Irradiation Apparatus



1. Lamp
2. Samples on table
3. Dark controls
4. Table
5. Water bath

Fig. 20

Dark controls were kept in a metal box, beside the black painted table supporting the irradiated samples. The table top was level with the water surface but not flooded. A laboratory thermal equipment water bath was used, and the temperature kept at 37°C. Fig. 20 shows a diagram of the irradiation apparatus.

At the end of irradiation, all samples were placed in the darkbox for 30 minutes and then agitated after this. The fluid was poured into centrifuge tubes which were then closed and covered with foil for light-proofing. The samples were spun and brought back to the darkroom when Drabkin's reagent was added to an aliquot of each supernatant. This converted all haemoglobin to cyanmethaemoglobin, which could be quantified by spectrophotometry (blank : buffer + Drabkin's; 0% haemolysis : buffer + blood + Drabkin's; 100% haemolysis:

distilled water + blood + Drabkin's) at 420 nm. The results were expressed as percentage haemolysis.

	Absorbance	% Haemolysis	
	0.02	0	Calibration
	0.82	100	
Sample	0.42		

$$\text{Sample \% Haemolysis} = \frac{(0.42 - 0.02) \times 100}{(0.82 - 0.02)}$$

Potassium loss from cells due to haemolysis was also assessed by measuring free potassium in the supernatant, after centrifuging. The samples for haemolysis and those for potassium measurement were taken at the same time. The samples were sealed until they were assessed by a calibrated flame photometer.

(c) Results

Table 26 expresses the results of haemolysis experiments with benzanthrone including potassium release measurements. It is clear that negligible haemolysis occurred with the control and lowest concentrations, however, the cells lost considerable amounts of potassium with 4.343×10^{-7}M benzanthrone and 60 minutes irradiation. The standard error is large, but the loss is significant. The 4.343×10^{-6}M solution caused negligible haemolysis in darkness, and with 7 minutes irradiation, with a strong photohaemolysis after this, but the potassium loss occurred earlier than haemolysis reaching approximately 90% while the haemolysis was only 14%. The haemolytic effect of benzanthrone at a concentration of 4.343×10^{-6} was greater than with the highest concentration of benzanthrone (4.343×10^{-5}) in which dark haemolysis was possibly significant. It is probable that the stronger solution simply absorbed much of the incident radiation before it reached the cells, damping the lytic effect. The potassium loss was also reduced.

HAEMOLYSIS AND POTASSIUM-RELEASE EXPERIMENTS WITH BENZANTHRONE

	Irradiation Time (mins)	Mean Percent Haemolysis	Standard Error	Mean Percent Potassium Release	Standard Error
EtOH	Dark Control	3.2	3.1	16.6	7.0
	7	0.2	0.2	13.2	3.6
	20	0.7	0.2	13.5	2.3
	60	0.6	0.2	15.2	3.2
Benzanthrone (4.343×10^{-7}M)	Dark Control	0.2	0.1	19.4	4.7
	7	0.2	0.2	11.8	2.4
	20	0.2	0.2	16.7	2.5
	60	1.1	0.7	74.9	23.3
Benzanthrone (4.343×10^{-6}M)	Dark Control	0.5	0.2	11.8	2.6
	7	0.1	0.1	23.0	6.8
	20	14.0	6.4	89.5	17.4
	60	81.8	5.7	119.6	9.2
Benzanthrone (4.343×10^{-5}M)	Dark Control	9.1	5.1	22.0	2.6
	7	11.8	1.0	40.0	19.7
	20	11.4	0.7	50.4	10.2
	60	48.4	11.3	116.5	13.7

Table 26

4. RESULTS AND CONCLUSIONS

Daniels (1965) first described the photosensitized killing of a yeast, Candida albicans. The test was inconclusive in his hands as a number of known sensitizers gave negative results. Benzanthrone, however, gave positive results in this study.

It is clear from the results that benzanthrone phototoxicity also caused disruption of erythrocyte cell membranes, possibly by a colloid-osmotic effect. During the pre-lytic changes, there was a net potassium loss and the use of potassium monitoring with photohaemolysis provided a sensitive assay of membrane damage in this system.

Previous research into photohaemolysis indicates that the mechanism is most likely a two stage one. The first stage is variable; but initiates the secondary damage which is osmotic in nature, and probably similar in most types of photohaemolysis.

The primary damage may be oxygen-dependent, oxygen-independent, or a mixture (Cook & Blum, 1959). These are distinguished by the effects of oxygen deprivation and the use of reducing agents (Davson & Ponder 1940; Kochevar & Lamola 1979; Cook & Blum, 1959 and others).

Oxygen dependent photohaemolysis is also known as "photodynamic haemolysis" and was long held to be the only type. Cook & Blum (1959) and Swanbeck et al (1974) presented two models for primary processes of oxygen dependent photohaemolysis.

Swanbeck et al were able to demonstrate that photohaemolysis produced by kynurenic acid and UV in the range 350 - 380 nm was almost certainly due to singlet oxygen.

Several cases of oxygen-independent photohaemolysis have been found. Chlorpromazine and protriptylene cause oxygen-independent

photohaemolysis by the action of photo products which may disrupt the structure of the erythrocyte membrane by becoming incorporated (Johnson, 1974; Kochevar & Lamola 1979).

Chapter V11

MUTAGENESIS OF BENZANTHRONE

Including

1. INTRODUCTION
   (a) Ames Test

2. AMES TEST : IN VITRO TEST METHODS
   (a) Benzanthrone
   (b) Light Sources
   (c) Dilution of Benzanthrone
   (d) Terminology
   (e) Benzanthrone Photoactivation Experiments

3. RESULTS AND CONCLUSIONS

1. INTRODUCTION

(a) Ames Test

Ames (1971) described a sensitive and simple bacterial test for detecting possible chemical carcinogens and mutagens.

Mutants of Salmonella Typhimurium LT-2 have been derived which are sensitive to different classes of mutagens and have been used for the detection of chemical mutagens. The particular Salmonella Typhimurium strain LT-2, used by geneticists world wide, is not very virulent. Five strains are used at the Toxicological Laboratories of I.C.I.

Salmonella Typhimurium		TA 1535
"	"	TA 100
"	"	TA 1537
"	"	TA 1538
"	"	TA 98

These strains have a deleted excision repair mechanism which renders the organism incapable of DNA excision repair. These mutant strains will not grow on media which does not contain histidine. If these bacteria are treated with a mutagen, then by chance, sooner or later, there will be a mutation which will replace the abnormal base in the histidine gene and it will become functional again. The bacteria in which this mutation has occurred will now grow in histidine - free medium, and this forms the basis of the test.

It is assumed that such chemicals which produce a significant increase in the frequency of reverse mutation, as recognised by the ability of the cells to form colonies in the absence of adequate supplies of histidine, will have a high probability of being carcinogenic to the mammalian species.

Ames et al (1975) described the general methods for using the Salmonella Typhimurium test as a mutagenesis screening system. It is a rapid and economical test system which can be used to pin-point potentially dangerous chemicals among the thousands to which humans are exposed.

Several studies have been reported in which these in vitro systems have been used to evaluate carcinogenic and non carcinogenic chemicals. Purchase et al (1976) evaluated 120 compounds, 50% of which were carcinogens, in four in vitro (of which Salmonella Typhimurium was the only micro-organism test) and two in vivo test systems. The Salmonella system accurately predicted 91% of the carcinogens and 93% of the non carcinogens. The compounds tested were identified chemically as polycyclics, arylamines and alkylating agents.

Unfortunately there are a few mutagenic non carcinogens that give false positives, and some of these of potential use could be unnecessarily restricted from the market place. Purchase et al (1976) considered that some false positives were inevitable but conversely data also indicated a substantial number of false negatives.

While it is known that a number of known carcinogens are mutagens it is still unclear what percentage of human or animal carcinogens are actually bacterial mutagens. The in vitro systems can be applied to make guarded judgements for possible risks and for the selection of compounds for more in-depth evaluation in intact animals. Coupled with animal experiments, the in vitro systems can add a valuable dimension to the testing strategy of hazard evaluation in man.

Having ascertained from previous experiments the wavelengths of light responsible for inducing the phototoxic reactions to benzanthrone I decided to conduct a series of photo activation experiments involving benzanthrone in the Ames test on account of obvious human and commercial connotations.

2. AMES TEST : IN VITRO TEST METHODS

(a) Benzanthrone

Benzanthrone was tested during the validation of the I.C.I. Central Toxicological Laboratories short term carcinogenicity screening system and an Ames test carried out following the protocol of Longstaff (1978) was negative.

(b) Light Sources

In the photoactivation experiments ultra violet radiation was produced by 2 x 20 W blue fluorescent tubes arranged at a distance of 14.5 cm from the plates. The irradiation time being 60 minutes at a temperature of 37°C.

(c) Dilution of Benzanthrone

In the photoactivation experiments benzanthrone was dissolved in 95% ethanol to give solutions of 2.5 mg/ml; 250 µg/ml and 25 µg/ml. All the work involved with the benzanthrone solutions (except for the UV radiation itself) was carried out in dark or safe-light conditions.

(d) Terminology

S-9 fraction is an integral part of the Ames mutagenic test for detecting potential carcinogens. The term S-9 is widely used as the supernatant from centrifuging homogenised liver at 9000 x g. This fraction is also referred to as post mitochondrial supernatant, as centrifuging at 9000 x g removes unbroken cells, nuclei, mitochondria, lysosomes and peroxisomes.

(e) Benzanthrone Photoactivation Experiments

Ames Test Protocol (Longstaff 1980)

Method

Salmonella Typhimurium TA 100 and TA 98 were incubated in Oxoid

No.2 nutrient broth on a shaking bench for 17 hours at 37°C. 0.2 ml of this culture was then dispersed into sterile Bigoux bottles. 0.2 ml S-9 mix and 0.09 ml buffered isotonic saline were then added followed by 0.01 ml of the benzanthrone test solution just before blue light irradiation. Non irradiated Bijoux bottles were incubated in the dark at 37°C for the same period of time.

After irradiation/incubation, 2.0 ml soft agar was then added to the Bijoux bottles and the resultant mixture was then poured onto Vogel-Bonner base plates and allowed to gel. When the overlay had set, the plates were inverted and incubated at 37°C in the dark for 72 hours before counting.

Following the incubation period the plates were examined for lack of microbial contamination and evidence that the test was valid. The plates were counted by an automatic colony counter (Biotran), with the discriminators adjusted to a standard setting which only permitted the counting of mutant colonies. With the test substance plates there would ideally be evidence of toxicity at the highest dose, but, for non-mutagens a ratio of test to control reversion frequency of about unity should exist. For a compound to score a positive response there should be at least a two fold increase in reversion rate and evidence of a dose response relationship.

Diagnostic mutagens - 4 nitroquinoline-N-oxide and 2 amino-anthracene - as appropriate chemical controls were tested concurrently as per the standard Ames assay; each test concentration was tested in triplicate or duplicate. (Tables 27 and 28).

3. RESULTS AND CONCLUSIONS

The non-irradiated chemical controls (2 amino anthracene with S-9 (Plate 14), 4-nitroquinoline-n-oxide without S-9; and N-methyl-n-nitro-n-nitrosoguanidine without S-9 (Plate 15), show that both strains of Salmonella Typhimurium TA 98 and TA 100 were responding within the expected sensitivity ranges. The benzanthrone blue light irradiated plates show that there is a metabolic dependent activity associated with the chemical itself. Although as tested here, the response obtained does not meet the accepted criteria for a "positive" (de Seers & Shelby 1979), retesting at concentrations above 25 µg/plate would most likely produce a dose related increase in the spontaneous mutation rate in both TA 98 and TA 100 (and possibly other strains as well).

Comparison of the results from the ethanol control plates with and without light exposure shows that the blue light treatment has induced no significant increase in the spontaneous mutation rate. (Tables 27 and 28). This is important, in that any such induced effects would tend to mask any increase caused by other effects e.g. the benzanthrone photoproducts.

The results obtained with the light exposed culture/benzanthrone/S-9 activation system mixtures (Plate 16) show no significant increase in mutagenic activity as against that produced by non-irradiated benzanthrone (Plate 17), although the results with TA 100 + S-9 in particular support the dose related nature of the activity that is observed. The lack of increase could be due to two factors:

(1) Any photo-products produced are not inherently "more" mutagenic in the tester strains used.

(2) The blue light treatment used did not affect the benzanthrone.

The apparent increase in toxicity observed in plates containing UV treated benzanthrone in the absence of S-9 would tend to rule out the second factor.

AMES TEST

BENZANTHRONE PHOTO-ACTIVATION EXPERIMENT

DATE TESTED 27TH JANUARY, 1981.

DATE COUNTED 30TH JANUARY, 1981.

STANDARD PLATE		
PLATE	±	COUNT
1000		1013

STRAIN TA 98 + S-9	UV IRRADIATED PLATE							
DOSE µg/PLATE	PLATE 1	PLATE 2	PLATE 3	PLATE 4	PLATE 5	MEAN	S.D.	RATIO TEST/CONTROL
BENZANTHRONE								
25.0	55	43	54			50.7	6.7	1.60**
2.5	33	33	48			38.0	8.7	1.20
0.25	35	37	47			39.7	6.4	1.25
ABSOLUTE ETHANOL	32	30	33			31.7	1.5	
STRAIN TA 98 + S-9	POSITIVE CONTROLS NON IRRADIATED							
2 AA								
100	2525	2312				2418.5	150.6	106.07***
4.0	891	1934				1412.5	737.5	61.95***
0.16	163	145				154.0	12.7	6.75***
DMSO	18	23	21	23	29	22.8	4.0	
STRAIN TA 98	NON IRRADIATED BENZANTHRONE CONTROLS							
+ S-9	25.0 µgBZA	39	46			42.5	5.0	1.57*
	ETHANOL	27	27			27.0	-	
CULTURE SOFT	AGAR ONLY	22	17			19.5	3.5	
- S-9	25.0 µgBZA	24	19			21.5	3.5	0.83
	ETHANOL	20	32			26.0	8.5	
STRAIN TA 98 - S-9	UV PLATES IRRADIATED							
BENZANTHRONE								
25.0	10	20	13			14.3	5.1	0.61
2.5	18	12	18			16.0	3.5	0.68
0.25	16	14	16			15.3	1.2	0.65
ETHANOL	18	27	26			23.7	4.9	
STRAIN TA 98 - S-9	POSITIVE CONTROLS NON IRRADIATED							
4NQO								
4.0	203	173				188.0	21.2	11.46***
0.8	245	300				272.5	38.9	16.62***
0.16	70	43				56.5	19.1	3.45***
DMSO	13	16	23	20	10	16.4	5.2	

4 NQO = 4-Nitroquinoline-N-oxide) Diagnostic Mutagens
2 AA = 2 Aminoanthracene)
DMSO = Dimethyl Sulphoxide - Solvent for positive control
BZA = Benzanthrone

*** $P < 0.005$
** $P < 0.01$
* $P < 0.05$
Students t test

Table 27

AMES TEST

BENZANTHRONE PHOTO-ACTIVATION EXPERIMENT

DATE TESTED 27TH JANUARY, 1981.

DATE COUNTED 30TH JANUARY, 1981.

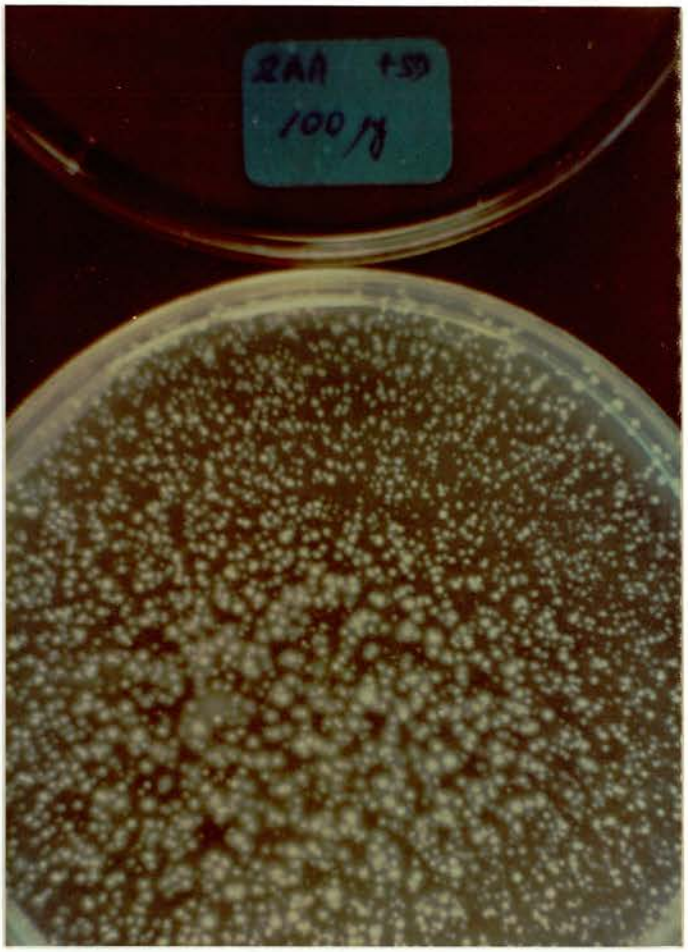
STANDARD PLATE		
PLATE	±	COUNT
1000		1013

STRAIN TA/100 + S-9	UV IRRADIATED PLATE							
DOSE µg PLATE	PLATE 1	PLATE 2	PLATE 3	PLATE 4	PLATE 5	MEAN	S.D.	RATIO TEST/CONTROL
BENZANTHRONE								
25.0	303	246	232			260.3	37.6	1.65*
2.5	239	173	181			197.6	36.02	1.23
0.25	173	159	166			166.0	7.0	1.05
ABSOLUTE ETHANOL	158	150	166			158.0	8.0	
STRAIN TA 100 + S-9	POSITIVE CONTROLS NON IRRADIATED							
2 AA								
100	2369	2849				2609.0	339.4	23.68***
4.0	3050	2746				2898.0	215.0	26.30***
0.16	301	323				312.0	15.6	2.83***
DMSO	111	121	99	96	124	110.2	12.6	
STRAIN TA 100	NON IRRADIATED BENZANTHRONE CONTROLS							
+ S 9	25.0 µgBZA	263	270			266.5	5.0	1.88***
	ETHANOL	138	146			142.0	5.7	
CULTURE SOFT	AGAR ONLY	115	141			128.0	18.7	
- S 9	25.0 µgBZA	147	137			142.0	7.1	1.06
	ETHANOL	130	139			134.5	6.4	
STRAIN TA 100 - S-9	UV PLATES IRRADIATED							
BENZANTHRONE								
25.0	50	65	82			65.7	16.0	0.45
2.5	55	46	43			47.3	5.1	0.33
0.25	65	66	60			63.7	3.2	0.44
ETHANOL	127	169	138			144.7	21.8	
STRAIN TA 100 - S-9	POSITIVE CONTROLS NON IRRADIATED							
MNNG								
4.0	2566	2460				2513.0	75.0	30.42***
0.8	79	122				100.5	30.4	1.22
0.16	100	103				101.5	2.1	1.23**
DMSO	84	74	84	84	87	82.6	5.0	

MNNG = N-Methyl-N'-nitro-N-nitrosoguanidine } Diagnostic Mutagens
2 AA = 2 Aminoanthracene
DMSO = Dimethyl Sulphoxide - Solvent in positive control
BZA = Benzanthrone

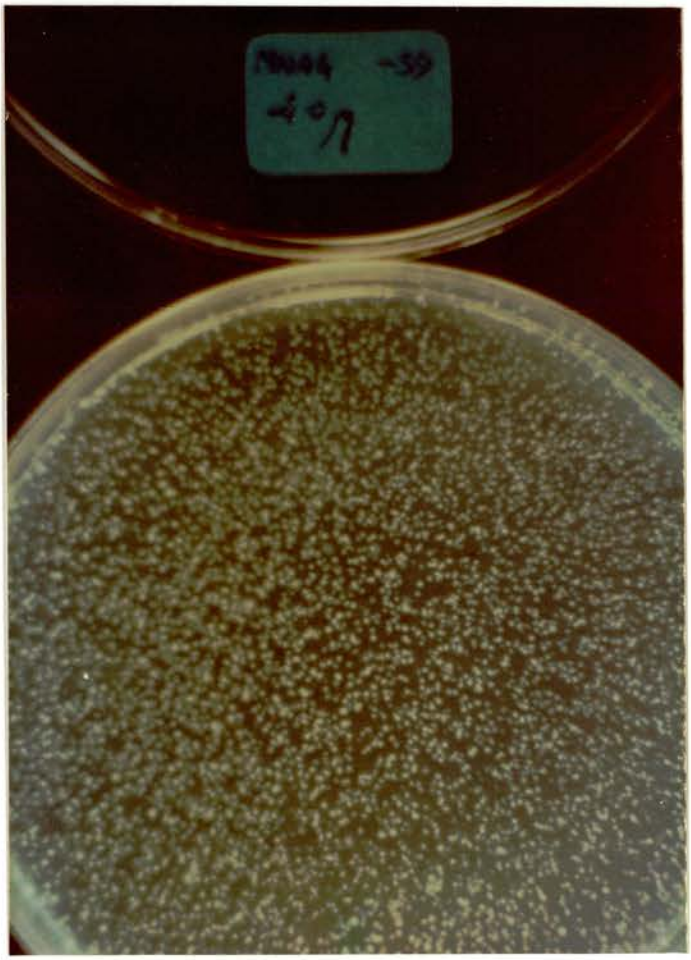
*** P< 0.005
** P< 0.01
* P< 0.05
Students t test

Table 28



2 Amino Anthracene + S-9. (Non irradiated chemical control). A diagnostic chemical mutagen showing profuse growth of TA 100 + S-9 Salmonella Typhimurium at a concentration of 100 µg/plate.

Plate 14



N-Methyl-N-Nitro-N-Nitrosoguanidine - S-9. (Non irradiated chemical control). A diagnostic chemical mutagen showing profuse growth of TA 100 - S-9 Salmonella Typhimurium at a concentration of 4.0 µg/plate

Plate 15

Irradiated benzanthrone showing no significant growth increase in TA 100 + S-9 Salmonella Typhimurium at a concentration of 25.0 µg/plate

Plate 16

Non irradiated benzanthrone showing no significant increase in growth of TA 100 + S-9 Salmonella Typhimurium at a concentration of 25.0 µg/plate

Plate 17

Chapter V111

GENERAL DISCUSSION

Including

1. INTRODUCTION

2. ACTION SPECTROSCOPY

3. IN VITRO STUDIES

4. PREVENTION OF PHOTOTOXIC DERMATITIS

5. LIGHT SCREENING AGENTS

6. ASCORBIC ACID STUDIES

7. AMES MUTAGENICITY TEST

1. INTRODUCTION

Benzanthrone, an anthraquinone derivative, is the starting product for an important group of vat dyes and has, over many years, given rise to problems of skin burning, itching and erythema on exposure to bright sunlight, particularly in workers on the morning shift. It is not a serious clinical problem but represents a serious social problem in that workers cannot follow outdoor pursuits when affected.

It is noteworthy that the workers in Grangemouth Works involved in the benzanthrone manufacturing process experienced similar symptoms as the tar and pitch workers described in the historical review of photobiological activity in Chapter 2.

Despite the findings of Trivedi & Niyogi (1968) and Singh & Zaidi (1969) of "blackening of the skin" and "pigmentation" in benzanthrone workers in India, none of the exposed workers in Grangemouth have experienced these signs, even after many years exposure. This is probably due to differences in racial characteristics.

In the patch and photopatch investigations, subjects were selected with differing working exposure times to benzanthrone. Some were constantly in contact with the chemical and therefore could conceivably develop a 'photoallergic' response. However, no differences in reaction were elicited between subjects in constant contact with benzanthrone when compared with workers not previously exposed to benzanthrone. This gave a good indication that photo-toxicity was involved. In one case, one employee (Plate 3, Page 64) developed a rare non photosensitive contact dermatitis, but this is a different problem.

Under the controlled conditions of phototesting, photosensitivity reactions with an early erythema phase were obtained. Latterly, with monochromator testing, subjects also developed burning sensations, so that, overall, all the facets of the clinical problem were demonstrated.

The findings of this thesis have elucidated some of the mechanisms of benzanthrone phototoxicity. At present, it seems unlikely, that there is a simple means of influencing the chain of events, and that prevention of benzanthrone phototoxicity will require the full co-operation of both the work force and the management.

2. ACTION SPECTROSCOPY

The absorption spectrum of the photosensitising molecule usually determines the action spectrum of photosensitization, but in a heterogeneous system such as skin, the action spectrum may be greatly modified either by binding of the agent with cellular constituents, or by preferential localization of the agent in certain layers or by metabolic conversion of the compound. An agent that has been applied topically, and is lodged on the surface of the stratum corneum, may act as an innocuous or even protective light screen; the same agent may prove to be a potent sensitizer however, if it reaches the viable Malpighian cell layer via the extra cellular fluid after ingestion.

Ultra violet radiation with wavelengths less than 300 nm does not penetrate the human epidermis deeply, especially in the exposed areas as face, hands and neck. Most of these short waves are absorbed and scattered by the stratum corneum. On the other hand ultra violet radiation with wavelengths greater than 300 nm can penetrate to the viable epidermis and even to the dermis; because the longer wavelength radiation penetrates better into the skin, the chances of an abnormal photosensitivity reaction occurring when a photosensitizer is present which specifically absorbs these longer longer wavelengths are higher. Hence, one would expect many cutaneous photosensitization reactions to be induced by radiation with wavelengths of 300 nm or more.

The results of extensive patch and photopatch testing, using the Xenon light sources and cut-off filters described in Chapter 4, show that the action spectrum for benzanthrone phototoxicity peaks around 390 nm, which matches the absorption spectrum of benzanthrone.

3. IN VITRO STUDIES

Both *in vitro* tests show that a phototoxic reaction is possible and therefore represent an extra indication that the reaction seen in the skin is phototoxic. It is clear from the *in vitro* work described in Chapter 6 that the photohaemolysis indicates that the primary target for the response may indeed be some part of the cell membrane complex, just as it is for protoporphyrins, anthracene and Rose Bengal. This response may indicate that the reaction is "photodynamic" - oxygen dependent, but no time was available to demonstrate this. The site of action is hypothetical, but the mast cell membrane might be involved and therefore anti-histamines might prove of value in treatment. The *in vitro* indication of a membrane effect which may be oxygen dependent might be expanded to phototesting of the skin of the lower arm; a sphygmomanometer cuff being used to cut off the oxygen, and observing whether the skin reaction is oxygen dependent. This also fits with the immediate type of reaction obtained in the skin.

The *in vitro* work on Candida albicans shows that benzanthrone is phototoxic. The target here is uncertain, but DNA may be involved. The psoralen type of skin photosensitive reaction is not obtained with benzanthrone.

In the study of industrial phototoxicity problems, a diagnostic approach using preliminary *in vitro* screening to establish phototoxic potential followed by limited *in vivo* testing would appear to have merit.

4. PREVENTION OF PHOTOTOXIC DERMATITIS

Interestingly, Koelsch as long ago as 1926 stated that exposure to tar and pitch might produce "acute eczema" aggravated by exposure to light. He recommended that processes which exposed workers to the action of photosensitizing substances should only take place at night and further advocated regular changes of clothing, washing after work and applications of mud or clay to the face. In theory, restricting manufacture of benzanthrone to the night shift is attractive, but in commercial terms is unrealistic due to the demands of a modern and highly competitive dyestuffs industry.

The significance of the investigations carried out by Trivedi & Niyogi (1968) is important as undoubtedly the extremely high levels of benzanthrone dust in the atmosphere was a major causative factor in the aetiology of the burning and pigmentation suffered by their workers. Similar analysis for benzanthrone dust present in the atmosphere of the manufacturing plant in Grangemouth has been investigated on many occasions. Air sample analysis showed that the total benzanthrone dust present in the plant was 2.83 mg/m^3 and at the bandcaster, which is the most likely area for dust to occur, the value was only 1.16 mg/m^3. These values are satisfactory and well within the threshold limit values for nuisance dust of 10 mg/m^3. Observations over many years have shown that frequent application of water to the floors of the manufacturing plant lessens the amount of free benzanthrone dust contaminating the atmosphere and thereby reduces the incidence of symptoms.

In addition, employees at Grangemouth are supplied with cleanly laundered underwear, socks and boiler suits at the start of each shift and full showering and changing facilities are also available.

5. LIGHT SCREENING AGENTS

Incorrect assumptions have been made in the past that light screening agents are of little real practical value in the treatment of photodermatitis. It is now appreciated that the arbitrary choice of a light screening agent, without first of all correlating its absorption spectrum with the action spectrum responsible for the skin reaction, is unsatisfactory. However, with the advent of equipment like the diffraction grating monochromator (Mackenzie & Frain-Bell, 1973) and its application to the study of action spectra in patients with photosensitivity eruptions, the choice of light screening agents has been put on a more satisfactory basis.

The function of sun screening agents is to absorb light and thus to protect the skin from injurious radiation. These are of two main categories (a) chemical and (b) physical. The principal chemicals used topically to screen short wave ultra violet sunburn wavelengths below 320 nm are para-aminobenzoic acid and its esters, benzophenones (mexenone) and cinnamates. The physical sun screening agents form an opaque barrier to solar radiation without absorbing such rays. Titanium dioxide scatters and reflects light and thereby acts as a physical barrier to its penetration of the skin. However, its opaqueness makes the sun screen visible and masklike, but it has the advantage over chemical sun screening agents of not producing allergic or photoallergic reactions.

In a study by Macleod & Frain-Bell (1975) of the use of titanium dioxide in a variety of formulations using *in vitro* techniques it was shown to be effective throughout the wavelengths 400 - 700 nm. It was also shown to have a satisfactory protective capacity in those photodermatoses in which the action spectrum involves wavelength peak

365 nm (335 - 395 nm) and 400 nm (370 - 430 nm).

Titanium dioxide has been used in the paint and ceramic industries for years because of its light scattering properties, and Calnan (1963) reported its use as a covering cream in skin blemishes. It has been said to possess a reasonable cosmetic acceptability provided care is taken to incorporate in the formulations colouring agents, which can be varied to suit the individual patient. The colouring agents (red ferric oxide and solution of burnt sugar) used for cosmetic purposes selectively absorb some of the light scattered by the titanium dioxide. Whether such a colouring agent based on titanium dioxide will be acceptable to the workers at Grangemouth, despite proven in vitro efficacy, will be the subject of future investigations.

6. ASCORBIC ACID STUDIES

Chapter 5 describes the investigations relating to photopatch testing after systemic administration of mega dose ascorbic acid. Statistically, and subjectively for the workers, the results are highly significant in the amelioration of the symptoms due to benzanthrone. In practical terms however, it may well be that the dose of ascorbic acid required to produce a reduction of symptoms is too high for regular prophylatic use; as it was, I had difficulty in persuading the workers to take the tablets for the two week period of the investigation due to various complaints such as diarrhoea and sore tongue.

A trial of beta-carotene is another possibility as this has been shown to be effective in some cases of erythropoetic protoporphyria. The signs and symptoms of this condition are similar in some respects to those of benzanthrone phototoxicity, though whether the workers would accept the yellow skin staining due to carotenaemia is questionable.

7. AMES MUTAGENICITY TESTING

Current scientific consensus is that exposure to occupational chemicals may be the underlying cause of some one to five per cent of cancer deaths occurring today. A few causes have been found by epidemiological investigations, but some have been found first by animal tests. Such tests show that there are great differences in the potency of different carcinogens. However, in spite of the evidence that chemical substances are important causes of cancer in man, the active substances are not easy to identify. Many of the causes of cancer in man have been found by the investigation of occupational disease and this is an approach that is still most promising.

Because the actual chemicals causing cancer are difficult to identify by examination of human populations it is essential to carry out animal tests and *in vitro* bacterial tests on chemicals in the environment in order to obtain indications of carcinogenic activity. The Ames test is one such test.

Brown & Brown (1976) reported that ninety substituted anthraquinones were submitted to the Ames test and that one third were reported as positive. As a result of this, a three year retrospective epidemiological mortality survey was commenced at Grangemouth Works (Gardiner, Walker & McLean, 1982) which showed that there was not an abnormal number or pattern of deaths in Grangemouth Works, and that while laboratory studies and animal studies on anthraquinone compounds were of interest, their results were not reflected in the health of workers producing anthraquinone dyes.

As a result of the study reported by Brown & Brown (1976)

benzanthrone was tested during the validation of the ICI Central Toxicological Laboratories short term carcinogenicity screening study using the Ames test, and was proved to be negative.

However, despite the reassurance of the negative Ames test, I decided to amplify this test by using irradiated benzanthrone. These modified Ames tests were again negative, and therefore are of significant relevance to the work force on the manufacturing plant.

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